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INSIDE SCIENCE

The Vital Story of
WHITE FLOUR ENRICHMENT

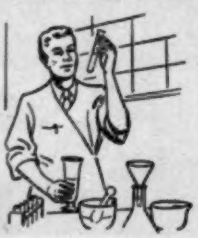
by Science Writer

The blessings of better health and increased vigor are in store for many Canadians as a result of the start of flour enrichment by Canadian millers. Dramatic results in improving public health through the use of enrichment have been shown by experience in the United States, the Bataan peninsula in the Philippines, and Newfoundland. The benefits have been so outstanding in these and other nations that more and more countries are adopting enrichment.

1953 sees the enrichment of white flour put into practice throughout Canada. This program became possible through the recent amendment of the Food and Drugs Act.

Everyone in the milling industry knows that the great majority of Canadians, like other North Americans, want beautifully fine, white flour. When wheat is milled and processed to get this white flour which the public demands, vitamin and mineral values are unavoidably lost. The loss of essential vitamin and mineral elements is easily overcome by enrichment.

The enrichment of ordinary white flour is simple and inexpensive. It restores these vital vitamin and mineral factors to milled white flour: thiamine, riboflavin, niacin and iron. Calcium also may be added as an optional ingredient. Canadian specifications also provide for enrichment of higher extraction flour (Vitamin B White Flour, Canada Approved).



Technology is so advanced these days that scientists are able to "build" duplicates of many of Nature's essential complexes in the laboratory. This has happened with many vitamins. First, the chemical composition is learned and the pure substance is isolated. Then a "duplicate" is made which is identical with Nature's product chemically and in biological activity. A vitamin is a vitamin regardless of its source, just as salt is salt whether it comes from a mine or is evaporated from the sea. So efficient is large scale manufacturing that vitamins are sold at a lower cost than if they were extracted from natural sources.

These are the vitamin and mineral factors which are used in white flour enrichment:

Thiamine—also called vitamin B_1. This vitamin helps to build physical and mental health. It is essential for normal appetite, intestinal activity and sound nerves.

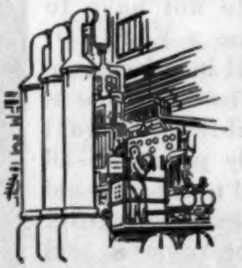
Riboflavin—also called vitamin B_2. This vitamin helps to keep body tissues healthy and to maintain proper function of the eyes. It is essential to growth.

Niacin—another "B" vitamin, is needed for healthy body tissues. Its use in diet is effective in controlling the serious disease, pellagra.

Iron—is the mineral used in enrichment. It is essential for making good red blood and preventing nutritional anemia.

To meet the amended regulations of the Canadian Food and Drugs Act, each pound of *enriched flour* must contain:

	Not less than	*Not more than*
Thiamine	2.0 milligrams	2.5 milligrams
Riboflavin	1.2 milligrams	1.5 milligrams
Niacin or Niacinamide ..	16.0 milligrams	20.0 milligrams
Iron	13.0 milligrams	16.5 milligrams



The Hoffmann-La Roche people manufacture top quality vitamins literally by the tons. To do this they must use amazingly complex processes with scientific production controls and the latest equipment which fill a city block square and many stories high. The flour-enriching ingredients are later combined in a form called premix which flour millers add to flour manufacture so that the enrichment is spread evenly throughout the flour.

This article, reprints of which are available without charge, is published as a service to the milling industry by the Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul Street, West, Montreal, Quebec.

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THE SANITATION OF CANADIAN FLOUR MILLS AND ITS RELATIONSHIP TO THE MICROBIAL CONTENT OF FLOUR[1]

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ABSTRACT

A preliminary survey of 50 representative Canadian flour mills has been carried out in order to estimate the relationship between sanitation in the mill and the microbial and insect-matter content of the flour. The following determinations were made from the whole wheat, from flour and from stock at seven intermediary stations in the mill: whole insects, larvae, eggs; insect fragments visible macroscopically and microscopically at 30x and, for flour, at 100x magnification; bacterial plate counts at 37°C. and at 22°C.; mesophilic spores, "rope" spores; total fungi; total aerobic thermophilic spores, "flat-sour" spores, and anaerobic gas-forming thermophilic spores. A method for concentration of microscopic insect fragments from flour and estimation by use of the Howard Mold Count cell has been devised.

Mills that practiced a superior quality of sanitary control produced flour with insect and microbial values for most of the above categories that were much lower than the median values for all mills. Flour from the more heavily infested mills had about 7½ times as many microscopic insect fragments as the average values for all the rest and, bacteriologically speaking, was 5½ times dirtier. A many-fold disparity existed between values for "superior" and "sub-standard" mills. This was true for all categories except thermophilic spores, though was not invariably true for each specific mill.

Significant correlations based on data from all mills were found between the microscopic insect fragment count of flour and the following categories: (a) sum of the insect fragment counts from intermediary mill sites; (b) sum of excreta counts for intermediary mill sites; (c) mesophilic spores (including rope spores) in flour; (d) nutrient-agar plate count of flour; (e) total mesophilic micro-organisms of flour. The sum of insect fragments (30x) at intermediary mill sites was correlated with fungi in flour. Fungi in flour were also correlated with fungi on the whole grain; fungi at 1st break-rolls; fungi in low-grade and tailings boots and in middlings boots. Fungi in boots were correlated with insect fragments in boots which in turn, were correlated with insect excreta and total thermophiles in the same boots. Total mesophiles of one series of boots were correlated with insect excreta, and insect fragments in the same boots, and the insect fragments showed further correlation with whole insects and with larvac in the same boots. Mesospores were correlated with larvae in boots.

Insects and excreta found in boots were found to contribute some nu-

[1] Manuscript received May 21, 1952. Contribution from the Food and Drug Laboratory, Department of National Health and Welfare, Ottawa, Canada.

merically dominant species of microorganisms in flour which were not dominant in wheat. Microscopic evidence of the contribution of fungi in flour made by the wheat and by insects is presented. The significance of these findings is discussed analytically with regard to the influence of mill-practice and of environment within the mill upon the microbial and insect content of flour.

It is concluded that the degree of insect infestation of mills bears a general relationship to the presence of microscopic insect fragments and of certain categories of microorganisms, including those of spoilage significance, in the finished flour, and that conditions which allow for severe infestation of dead stock in elevator boots tend to emphasize this relationship.

This paper reports the outcome of a preliminary survey of the conditions of sanitation of Canadian flour mills. The purpose of the survey has been two-fold: (1) to determine the degree of insect infestation of a representative group of mills and the degree and type of microbial contamination at significant sites within the mills; (2) to determine any relationship which may exist between these factors and the microbial and insect-fragment content of the finished flour.

The particular groups of microorganisms investigated were those which have specific significance as a cause of spoilage in bakery products or in foods to which flour is added as an accessory ingredient, and also those which might serve as indicators of insanitary conditions. Thus, in the first category, spores of mesophilic bacteria have been determined because of their relationship to "ropiness" in bread and other products; molds have been determined because they also are a potential source of bread spoilage; thermophilic spore-bearing species are of significance both in bread and in secondary products such as canned soups, sauces, etc., while "total counts" may provide a general indication of degree of contamination.

The selection of mills was based upon three factors: (a) to provide a wide geographic distribution, (b) to provide a range of size in mills from very small to the largest in Canada, and (c) to include in each category mills which previously had had a record of efficient sanitation as well as those known to be subject to severe insect infestation.

Materials and Methods

Inspectors specially trained for the task examined as much of each mill as feasible within a time limit of one day per mill. They were specifically instructed to note visible traces of insect or rodent infestation in and about the following sites: elevator "boots", elevator "legs", rolls, roll-stand-hoppers, purifiers, spouts, bran-dusters, reels, sifters, conveyors, elevator heads, feed-in chutes and storage bins. From this inspection and after an appraisal of the attitude of the mill operators

towards maintaining consistently sanitary conditions the inspector tentatively classified a mill as "superior", "standard", or "substandard".

Samples for laboratory examination were collected from nine different sites within each mill, the intention being to reveal the more probable sources of any contamination which might appear in the finished product. The samples collected were as follows: (1) whole wheat kernels from the "stream" immediately before entering the first break-rolls; (2) from flour-stock (excluding "break-stock" and "feed" streams) after passing through a number of break-rolls; (3) from the "stream" at a later point in the mill operation, usually after passing through a "scalper", or a "sifter"; (4) "dead" stock (i. e., not in motion)–(a) from a boot in a low-grade stock line and (b) from a "tailings" boot, the two being mixed into one sample; (5) "dead" stock from a middlings boot; (6) from bran at the bran-packing machine; (7) from "shorts" at the "shorts"-packing machine; (8) final unbleached flour; (9) final bleached flour on entry into the flour-bag or the bagging machine.

Specimens were collected with clean, dry instruments and shipped in quart-size "Sealer"-type glass jars to a central labratory, via "express" on the same day that they were collected, and held in a refrigerator until examination was possible.

The laboratory determinations made with each sample were as follows: (1)Insect material–(a) whole adult insects and larvae; (b) macroscopically visible insect-fragments; (c) insect fragments visible at 30× magnification using a stereoscopic binocular microscope; (d) insect eggs; (e) insect excreta; (f) (for finished flour only) "microscopic" insect fragments detectable at 100× after a newly devised treatment to be described later; (2) microbial determination–(a) standard plate-counts for mesophilic bacteria using two different media; (b) the mesophilic spore-count; (c) the "total fungus-count"; (d) total aerobic thermophilic spores; (e) "Flat-sour" thermophilic spores; (f) anaerobic gas-forming thermophilic spores.

I. The Determination of Insects and Insect Residues.

(A) The Determination of Insect Material in Wheat Grain. Triplicate specimens of 100 g. of washed whole wheat kernels (specimen No. 1) were spread over a sheet of opalescent glass illuminated from below. The glass and lights, suitably mounted, will be referred to as the "viewing box". The whole-insects, fragments and eggs recognizable as such at a magnification of 10× were recorded. This brief ex-

amination was considered adequate because preliminary tests showed that the numbers of insect excreta and small fragments were negligible at this point in the stream. Also, Gray has reported that Canadian wheat contains relatively little internal insect infestation (9).

(B) The Determination of Insect Material in Flour "Stock" from the "Streams". The conventional oil-flotation method of the A.A.C.C. (2) was not found to be satisfactory for coarse specimens because a relatively large amount of bran and other coarse particles accumulated in the petroleum ether (or gasoline). These particles proved to be a great hindrance to accurate counting of the insect fragments. Accordingly, the method adopted was to sift 100 g. of the specimen progressively through 20-, 60- and 80-mesh sieves. The 20-mesh sieve retained most of the large particles that interfered with the flotation method, and also retained adult insects, larvae and the larger insect fragments. This latter group were counted by spreading the retained material, in small portions, over the glass in the "viewing box" previously mentioned.

The 60- and 80-mesh sieves retained most of the insect excreta. This was estimated by the A.O.A.C. method (4). The material retained by each sieve was weighed, then 0.2-g. portions were spread over a glass plate having an inscribed area of 4 x 5 in. The specimen was mixed with a few drops of low viscosity paraffin oil and examined at a magnification of 30×. In the event that very large numbers of fecal pellets were present then ten microscopic fields of finite area were examined and the number recorded was expressed in terms of the number present in the whole 4" x 5" area. The numbers of fecal pellets counted in each 0.2-g. specimen were expressed in terms of the total amount of material retained by each sieve. By summation of these two values, the number of excreta per 100-g. sample could be estimated. Insect eggs were counted from the same preparations using the "overs" from an 80-mesh sieve in the absence of an excess of other material. The residue of the material retained by the 60- and 80-mesh sieves was then added to the material passing through the 80-mesh sieve, the aggregate weighed, then subjected to a flotation procedure for recovery of small insect fragments. The method was a modification of A.A.C.C. (9), with accessory staining in accord with Gier, *et al.* (7) and the use of a separatory funnel as described by Gilbert, *et al.* (8).

The aggregate obtained from the various sieved fractions was well-mixed and 50 g. placed in a 600-ml. beaker, mixed into a slurry with water, then water added to a total of 400 ml. Hydrolysis of starch was accomplished by adding 17 ml. of conc. hydrochloric acid, stirring

continuously while heating to boiling, then boiling for 10 minutes with intermittent stirring. If the specimen is to be treated as in paragraph (D) fast violent stirring should be avoided. The use of fast electrical stirrers was found to be the factor most conducive to the formation of emulsions at later stages in the procedure. After cooling, the mixture was made slightly alkaline by adding, while stirring, 48 ml. of 20% sodium hydroxide solution. It was then subjected to the staining method of Gier (7) by adding 10 ml. of a 1% solution of azure I in methyl alcohol. The mixture was allowed to stand for 30 minutes then transferred quantitatively to a large separatory funnel. Fifty ml. of petroleum ether was added, shaken thoroughly, allowed to stand until complete separation of the two solvents was accomplished. Then the greater part of the aqueous layer was drained off. More water was added, re-shaken, separated and the aqueous layer again removed. This was repeated until practically no more blue stain diffused into the aqueous layer. The petroleum layer was then filtered through lined "sharkskin" filter paper in a Buchner funnel. The paper was washed *in situ* in turn with 20 ml. water, 10 ml. ethyl alcohol, 10 ml. acetone. When the paper was dry it was transferred to a Petri dish (6" diameter) and moistened with low-viscosity paraffin oil. The filter paper was examined at a magnification of 30×, insect particles usually being stained a greenish color, the fragments of plant origin being blue. Mites are frequently unstained but are morphologically recognizable. The number of small fragments per 100 gm. of original sample may be expressed by estimating the number present in the whole aggregate which provided the flotation specimen.

(C) *The Determination of Insect Material in "Bran" and "Shorts".* Whole insects, the larger insect fragments, excreta and eggs present in bran and shorts were determined by essentially the same methods as described for flour stream specimens (B above). However, when bran and shorts were treated by the flotation method so many interfering particles accumulated at the water-oil interface that the method was discarded for these materials. Instead, an additional sifting through a 10-mesh sieve was used to remove the larger bran particles, and, after the estimations had been made on subsequent sifted fractions of the sample, the residues of the material retained by the 60-mesh and 80-mesh sieves were added to the material passing through an 80-mesh sieve, mixed and an 0.5-g. specimen stained with 1 ml. of azure I solution, washed, filtered and the filter-paper examined at 30× magnification.

(D) The Determination of Insect Material in Finished Flour. As

might be expected, the freshly bolted flour rarely contained any whole insects and only few insect fragments detectable by the methods described above. Since one purpose of the investigation was to examine any possible relationship between insect fragments and microbial content, a more exacting method for the determination of insect fragments of microscopic size was devised. It was essentially a hydrochloric acid digestion followed by flotation as described previously. However, instead of filtering the petrol-ether layer as before, the aqueous layer was drawn off as carefully as possible, then the wettability of the insect fragments suspended in the petrol ether was reversed by adding 0.5 ml. of detergent solution (0.1% "Aerosol O.T." American Cyanamide Corp.). The specimen was then mixed gently for several minutes with a careful rotary motion to avoid formation of emulsions, the sides of the funnel wiped downwards with a rubber-capped stirring rod. After standing until the petrol-ether layer was clear, the aqueous layer, and a small amount of the petrol-ether, to clear the stop-cock, was run into a watch-glass. The petrol-ether was allowed to evaporate. Thus, the total insect-recovery was now concentrated in the 0.5 ml. aqueous suspension. The residual suspension was then thoroughly mixed by gently agitating with a stream of air from a fine-bore pipette. About 0.1 ml. of the suspension was drawn into a pipette and three non-consecutive drops placed on the surface of a Howard mold-count cell. A Howard coverslip was added and the preparation examined at 100× magnification. The numbers of insect-fragments detectable in each of 25 microscope-fields were recorded. In order to observe a representative selection of microscope-fields, only alternate fields were counted as the specimen was moved with the mechanical stage of the microscope. The counting procedure was carried out in quadruplicate and the average count per 25 fields determined. By measuring the diameter of the microscope-field, the volume subtended per field could be determined and, in turn, the number of fragments present in the 0.5-ml. suspension and, hence, the number in the original flour aliquot could be calculated. In the event that the specimen contained very little insect material, or if separation of the two phases was not accurately done, the specimen could be evaporated to dryness and taken up in 0.1 ml. water. The presence of the aerosol allowed ready dispersion and prevented absorption of particles on the surfaces of the pipette.

While staining is an aid in differentiation, the microscopist must be able to recognize the cellular nature of plant materials and should have become familiar with insect particles by having studied several

preparations of fragmented mill insects beforehand. Accuracy is improved by examination of dubious fragments at higher magnification.

II. Microbial Determinations.

(A) The Estimation of the Mesophilic Bacteria and Fungi Present in Flour-Mill Specimens. (i) *Bacteria: "total count".* Two different media were used to obtain an estimate of different segments of the total bacterial population. Using nutrient agar, viable counts were determined by the "drop-plate" method of Reed and Reed (12). Plates were incubated at 37°C. for 48 hours. The second medium, potato-dextrose agar, was incubated at 22°C. for 48 hours. The development of large mucoid colonies made the standard dilution procedure preferable for this latter medium.

Dilutions were prepared by suspending 1 g. of a thoroughly mixed sample in a 99 ml. dilution blank containing sterile water or buffer at pH 7.0 and shaking violently in a mechanical shaker for 5 minutes. (The use of buffer was discontinued after earlier tests failed to show any advantage). Progressive dilutions were similarly prepared. Plates of potato-dextrose agar were poured in triplicate from each appropriate dilution. The same dilution specimens were used to seed plates of solidified nutrient agar with calibrated drops from fine pipettes in accord with the drop-plate method. Dilutions were prepared for finished flour to $1:10^4$; for obviously dirty specimens to $1:10^6$. Three dilutions were usually selected for each specimen and plates prepared in triplicate. Where whole grain was the specimen, only the water in which it had been shaken was plated. This applies for all bacteriological procedures in this study.

(ii) *Bacterial spores.* The number of spores of mesophilic bacteria were estimated by heating diluted specimens prepared as described above to 80°C. in a water bath and holding them at that temperature for 10 minutes, then plating with "Difco" tryptone-glucose agar. The method described in Cereal Laboratory Methods (1) which provides for exposure to boiling temperature for 20 minutes was at first used concomitantly, but was discontinued since an unnaturally low count was obtained. The use of the lower temperature for destruction of vegetative cells was effective since microscopic examination revealed that only spore-bearing species developed subsequently. Any delay prior to testing the suspension could perhaps initiate the spore-germination process and hence provide a falsely low count at the higher temperature.

(iii) *Molds.* For the determination of fungi present in mill-speci-

mens the method of Christensen (6) was preferred, using the "malt-salt-agar" devised by that author. Dilutions were prepared as described above, plates poured in triplicate, incubated at 25°C. for 5 days and examined with a stereoscopic microscope at 30× magnification.

(B) The Estimation of Thermophilic Spore-Forming Bacteria in Mill Specimens. The methods used for the determination of thermophilic spores were based upon those used for sugar by the National Canners Association and which are described in A.O.A.C. Methods (5). Some modification was necessary owing to the difficulty in obtaining adequate distribution of flour particles in an agar medium. The procedures used were as follows:

(i) *"Total" aerobic thermophilic spores.* Samples weighing 20 g. were added to a sterile dilution bottle previously marked at the 100 ml. level. Sterile water was added to reach the 100 ml. mark. The bottle was then stoppered and shaken violently in a horizontal position for 5 minutes, using a mechanical shaker. With a sterile, wide-mouthed pipette, 10 ml. was transferred to a flask containing 100 ml. of melted "Difco" dextrose-tryptone-brome cresol purple agar. After thorough mixing, the medium was heated to boiling for 15 minutes, cooled to 55°C., re-shaken and distributed equally among five Petri plates. After the agar had solidified it was covered with a thin film of melted plain agar at 45°C. in order to prevent development of "spreading" colonies. When the plain agar had solidified, the plates were incubated at 55°C. for 48 hours in a humidified incubator. All colonies were counted. One-half the number developing on the five plates was expressed as the number of aerobic thermophilic spores per gram. The whole procedure was carried out in duplicate.

(ii) *Flat-sour spores.* The number of flat-sour spores present in mill specimens was estimated by counting the numbers of acid-producing colonies which developed on dextrose-tryptone agar plates prepared and incubated as described in the previous paragraphs. The development of a yellow "halo" around a colony due to indicator color-change was the criterion of acid production.

(iii) *Anaerobic gas-forming thermophilic spores.* Liver-veal agar ("Difco") was the medium used for detection of anaerobic thermophiles. After preliminary tests with the method described for sugar (5) the following modification was chosen as the more practicable in overcoming the difficulty of distribution of the specimen. Suspensions were prepared in water as described for aerobic thermophiles. A flask containing 100 ml. of melted liver-veal agar was inoculated with 20 ml. of the flour suspension, mixed carefully with a rotary motion to minimize

TABLE I

THE NUMBERS OF WHOLE INSECTS PER 100 G. OF "STOCK" AT DIFFERENT SITES IN THE MILL, FROM 5 "SUPERIOR" MILLS AND 5 "SUB-STANDARD" MILLS

Mill Sites	SUPERIOR MILLS					SUB-STANDARD MILLS				
	Mill No. 8	Mill No. 20	Mill No. 22	Mill No. 24	Mill No. 29	Mill No. 3	Mill No. 14	Mill No. 25	Mill No. 27	Mill No. 31
1. Washed grain in stream[1]	0	0	0	0	0	0	0	0	0	0
2. Flour stock after several break rolls	0	0	0	0	0	0	0	0	0	0
3. Flour stream after scalping	0	0	0	0	0	0	0	0	0	0
4. "Dead" stock from low-grade boots	4	3	9	4	4	15	303	72	5,530	3,224
5. "Dead" stock from middling boots	0	17	9	1	0	10,913	169	99	31,515	638
6. Bran (at the packers)	0	0	0	0	0	0	0	0	0	0
7. Shorts (at the packers)	0	0	0	0	0	0	0	0	0	0

[1] Just prior to 1st-break rolls, top grade.

TABLE II

THE NUMBERS OF INSECT FRAGMENTS PER 100 G. OF "STOCK" AT DIFFERENT SITES IN THE MILL FROM 5 "SUPERIOR" AND 5 "SUB-STANDARD" MILLS

Mill Sites	SUPERIOR MILLS					SUB-STANDARD MILLS				
	Mill No. 8	Mill No. 20	Mill No. 22	Mill No. 24	Mill No. 29	Mill No. 3	Mill No. 14	Mill No. 25	Mill No. 27	Mill No. 31
1. Washed grain in stream[1]	0	0	0	0	0	0	0	0	0	0
2. Flour stock after several break rolls	0	16	0	2	0	0	20	18	14	24
3. Flour stream after scalping	10	0	13	0	0	20	13	48	36	4
4. "Dead" stock from low-grade boots	64	11	120	62	94	1,707	1,811	1,534	3,931	598
5. "Dead" stock from middling boots	0	21	28	11	0	2,094	1,226	1,238	6,860	1,223
6. Bran (at the packers)	2	0	0	0	18	0	0	0	0	0
7. Shorts (at the packers)	0	0	0	0	0	0	0	4	4	10

[1] Just prior to 1st-break rolls, top grade.

the incorporation of air bubbles, then divided equally among five sterile tubes of wide (20 mm.) diameter to facilitate pouring. The objections to pouring a specimen into tubes is recognized but the blocking of even specially wide-bore pipettes seemed to justify it. Pipettes were useless with bran and shorts. The specimens were incubatd anaerobically in a brewer-jar for 48 hours at 55°C. Gas formation in the agar was considered a positive test. In the event that all tubes from a specimen were positive further dilutions were similarly tested at a later date. Multiple dilutions were not prepared in the first instance because of the tax on incubator-space caused by the brewer jars, and because, for many specimens, the number of anaerobic spores present was low. Most probable numbers were then estimated from appropriate tables (3). It was recognized that only a crude approximation is obtainable by this method.

Results

In all, 50 mills were visited. Laboratory tests were made from thirty-two which were actively milling wheat at the time of the inspection. The number of individual determinations made of insect-fragments and microorganisms was about 8,000. To avoid an unwieldly number of tables, complete data from only five "superior" mills and five "sub-standard" mills will be presented, together with summaries of other pertinent data.

"Superior" vs. "Sub-standard" Mills. Table I reports the numbers of whole insects per 100 g. "stock" recovered from the various test sites within each of five superior and five sub-standard mills. Mites and larvae are included in these numbers. The whole grain does not contribute a significant number of insects in either category of mill and early "streams" similarly remain practically free from insects. The two elevator "boots" tested, however, serve as reservoirs of insects in mills of each category, but whereas the boots of superior mills contain an average of five insects per 100 g. of contents, average value for the sub-standard mills is 5,200 per 100 g. A large proportion of this total population consisted of mites.

Table II shows that a similar relationship exists between the insect-fragments recovered from the various test sites of the superior and sub-standard mills. The wheat does not introduce any insect fragments. A small amount of insect material gains access to the earlier streams, but again the boots of the two categories of mills show marked differences. The boots of the superior mills contain an average of 41 insect fragments (detectable at 30$\times$ magnification), while the boots

TABLE III

THE EXCRETA COUNT PER 100 G. OF "STOCK" AT DIFFERENT SITES IN THE MILL, FROM 5 "SUPERIOR" MILLS AND 5 "SUB-STANDARD" MILLS

Mill Sites	SUPERIOR MILLS					SUB-STANDARD MILLS				
	Mill No. 8	Mill No. 20	Mill No. 22	Mill No. 24	Mill No. 29	Mill No. 3	Mill No. 14	Mill No. 25	Mill No. 27	Mill No. 31
1. Washed grain in stream	0	0	0	0	0	0	0	0	0	0
2. Flour stock after several break rolls	0	0	0	0	0	0	0	0	0	0
3. Flour stream after scalping	0	0	0	0	0	0	0	0	0	0
4. "Dead" stock from low-grade boots	1,090	2,050	6,525	1,050	100	24,300	2,322,000	202,800	14,875,000	16,000
5. "Dead" stock from middling boots	0	56,100	7,100	500	0	2,428,000	1,264,000	182,400	19,000,000	272,000
6. "Bran" (at the packers)	0	0	0	0	0	0	0	0	0	0
7. Shorts (at the packers)	0	0	0	0	0	0	0	0	0	0

of the sub-standard mills contain an average of 2,200 fragments per 100 g.

Table III compares the insect excreta recovered from superior and sub-standard mills. As might be expected, the distribution of excreta is in accord with the distribution of insects and fragments. No excreta were recovered from the wheat kernels but the boots of the superior and sub-standard mills contained respectively an average of 7,500 and 4,050,000 excretory pellets, per 100 g. of contents.

Table IV reports the microbial counts obtained from the bleached flour of the five superior and five sub-standard mills. The average numbers of mesophilic bacteria and fungi are consistently lower in the flour from the superior mills. The average nutrient agar plate count for these latter mills is 7,300 per g.; the potato-dextrose agar plate count is 3,300; mesophilic bacterial spores 95; and fungus count (malt-salt-agar) 2,200. The corresponding counts for sub-standard mills were, respectively, 825,000; 12,000; 600 and 11,000.

The presence of thermophilic bacteria did not appear to be influenced by the insect population of the mill. "Flat sour" spores averaged 10 and 4 per g., respectively, for the two mill categories; total aerobic thermophilic spores 11 and 13; anaerobic gas-forming spores 23 and 9 for superior and sub-standard mills, respectively.

Mill Insects and Microbial Count of Flour.—All Mills. The relationship between the distribution of insect material at the intermediary mill sites and the microbial content of flour is illustrated by the data summarized in Table V. The first column indicates the sum of insect-fragment counts per 100 g. for each of the intermediary sites. The data for excreta counts is presented similarly. The data for microscopic insect fragments and for microbial content refers to bleached flour. With the exception of the thermophilic bacteria, a general relationship seems to be suggested between the degree of insect infestation and microbial counts. However, it will be noted that for any specific mill such a relationship is not always consistent.

The extremely high excreta count in several mills is noteworthy. The "dead stock" in boots was the chief contribution to these large figures. Some of these high counts were obtained from mills that had been fumigated shortly before collection of specimens. If boots are not adequately cleaned out the progressive accumulation of feces clearly represents a major contamination source for a wide variety of microorganisms.

This observation is further substantiated by the data in Table VI, which summarizes all categories of insect and microbial content of

TABLE IV

THE NUMBERS OF MICROORGANISMS IN BLEACHED FLOUR FROM 5 "SUPERIOR" MILLS AND 5 "SUB-STANDARD MILLS"

Microbial Category	NUMBER OF MICROORGANISMS PER GRAM											
	SUPERIOR MILLS						SUB-STANDARD MILLS					
	Mill No. 8	Mill No. 20	Mill No. 22	Mill No. 24	Mill No. 29	Average	Mill No. 3	Mill No. 14	Mill No. 25	Mill No. 27	Mill No. 31	Average
Mesophiles												
Plate count (N.A. 37°C.)	6,000	22,000	4,700	700	5,300	7,300	4,040,000	23,000	33,000	26,000	1,300	825,000
Plate count (P.D.A., 22°C.)	3,000	7,000	7,000	700	3,000	3,300	48,000	3,000	4,000	5,000	700	12,000
Bacterial spores	10	100	17	200	150	95	150	50	2,500	200	50	600
"Molds"	6,000	130	130	400	4,500	2,200	18,500	7,000	16,000	4,000	300	11,000
Thermophiles												
Total aerobic spores	3	3	9	28	12	11	28	13	11	9	3	13
"Flat sours" spores	2	1	6	33	7	10	10	11	5	3	1	4
Anaerobic spores	0	23+	23+	23+	23+	18+	6	23+	0	0	0	6+

TABLE V

THE TOTAL COUNTS OF INSECT-MATERIAL AT CERTAIN MILL SITES AND THE NUMBERS OF BACTERIA IN THE FINISHED FLOUR OF EACH MILL

	Insect Counts per 100 g.			Bacterial Counts (per g.)[1]					
Mill No.	Insect Fragment Count sum of sites 2-7[2]	Excreta Count sites 2-7[2]	Microscopic Fragments[1]	Mesophiles[3]	Mesospores	Molds	Aerobic Thermophiles Spores	Flat Sour Spores	Anaerobic Thermophiles Spores
1	700	31,000	1,000	23,700	50	1,300	0	0	6
2	6,400	4,000,000	1,800	68,000	250	2,000	10	8	12
3	4,100	2,500,000	6,600	4,516,000	150	18,500	28	0	6
5	1,800	500,000	900	274,000	450	3,300	2	1	3
6	250	2,500	2,600	60,000	0	200	5	2	3
7	300	82,000	3,600	7,000	100	300	5	3	6
8	80	1,100	860	9,000	0	6,000	3	2	0
9	500	250,000	1,800	27,000	200	500	14	2	3
10	970	560,000	1,800	39,000	2,550	625	16	15	9
11	2,800	3,400,000	1,600	5,000	50	6,000	5	2	0
12	1,700	1,300,000	800	272,000	100	2,000	10	9	12
13	2,100	1,200,000	340	3,000	0	500	4	3	23+
14	3,100	3,600,000	1,200	26,000	50	7,000	15	11	23+
18	700	170,000	340	3,400	200	500	68	4	23+
19	1,700	290,000	4,400	15,000	3,600	80	3	6	23+
20	50	58,000	450	29,000	100	130	3	1	23+
22	160	14,000	700	7,700	20	130	9	6	23+
23	1,400	740,000	3,600	9,000	100	1,900	7	4	23+
24	75	1,500	700	1,400	200	400	28	32	23+
25	2,900	390,000	1,600	38,000	2,500	16,000	20	16	0
26	500	9,000	900	54,000	100	10,500	11	5	0
27	11,000	34,000,000	11,000	31,400	200	4,000	9	3	0
29	90	100	170	8,300	50	4,500	12	7	23+
30	580	3,500	230	700	50	1,500	6	1	0
31	1,900	290,000	1,400	2,000	0	300	2	1	0
32	3,700	16,000,000	900	8,700	150	280	6	3	0
33	720	100	110	4,700	200	480	11	8	20
34	2	100	0	23,000	0	150	3	1	0
35	260	490	340	6,900	100	630	10	5	0
36	330	200	0	83,000	50	880	8	6	6
44	60	0	60	174,000	50	0	27	21	6
50	190	300	1,000	13,000	50	260	6	5	0

[1] Finished flour, bleached.
[2] Sites 2-7 refer to intermediary points between the washed wheat and finished flour.
[3] Sum of counts from nutrient agar and potato-dextrose agar.

dead stock in "low-grade" boots. The dominant insects recovered in adult and larval forms from these sites were the confused flour beetle *(Tribolium confusum)* the flat grain beetle *(Laemophloeus pusillus)*; the Mediterranean flour moth *(Ephestia kueniella)*; the yellow meal worm *(Tenebrio molitor)*; the Cadelle *(Tenebrioides maurittanicus)* and mites *(Tyroglyphus* spp.).

Relatively high microbial counts are to be noted in most categories. It is evident that infested boots are an important source of fungi which would be strategically located to provide most effective dissemination of spores via carriers and air-currents throughout the mill. It should be noted that these counts represent only those microorganisms which were recoverable by standard dispersion methods which, for the most part, only wash off the surface of the excreta. It is probable that counts would have been much higher if the hard pellets of excreta had been pulverized to allow for complete dissemination throughout the plating suspension.

The Effect of Bleaching on the Microbial Content of Flours. In so far as possible, specimens of flour were collected from each mill both before and after bleaching.

Table VII compares the average microbial counts for all mills for bleached and unbleached flour. With the exception of thermophilic spores (noted for their marked resistance to injurious agents) the overall result indicates a reduction of the numbers of bacteria and fungi by a factor of about one-half for all categories. Since an average number may be unduly "weighted" by a few extreme values a supplement to this appraisal of the effect of bleaching is provided by the tabulation of the per cent of mills in which bleached flour showed a reduction in microbial numbers in the various categories (Table VII). As might be expected, spore-bearing bacterial species seem to be less affected by bleaches than are others. Several different bleaching agents were in use by the various mills. Our data suggests that the most effective was nitrogen-trichloride which is now legally invalid for use in Canada. This agent was considerably more effective than chlorine-dioxide, currently the more commonly used bleaching agent.

A Study of the Correlations between Selected Pairs of Insect and Microbial Data—All Mills. To aid in appraising the significance of the experimental findings from the point of view of the effect of insect infestation of the mill on the "filth" content of flour, correlation coefficients have been calculated from several pairs of different categories of measurements. Some selection in choice of data has been necessary

TABLE VI

THE MICROBIAL AND INSECT CONTENT OF DEAD STOCK IN LOW-GRADE "BOOTS"

Mill No.	Total Count N. A. (37°C.)	Total Count N.A. P. D. A. (22°C.)	Fungi	Meso-spores	Flat Sour Spores	Aerobic Thermophiles Spores	Anaerobic Thermophiles Spores	Whole Insects	Large and Small Fragments	Excreta	Dominant Species
1	20,000	32,000	17,000		18	46	23+	16	168	23,150	Conf. Fl. Bt.[1] Flat Gr. Bt.[2]
2	17,000	29,000	1,300		5	6	0	63	978	406,750	Conf. Fl. Bt.
3	4,240,000	1,560,000	22,000		3	7	12	15	1,707	24,300	Conf. Fl. Bt.
5	12,000	4,000	1,200,000		4	8	23+	73	967	276,300	Conf. Fl. Bt.
6	1,300		6,000		15	17	3	16	164	1,150	Conf. Fl. Bt.
7	47,000	48,000	114,000		3	4	0	51	142	44,520	Conf. Fl. Bt.
8	88,000	130,000	17,000		4	6	23+	4	64	1,090	Conf. Fl. Bt.
9	73,000	146,000	19,000		23	39	23+	37	248	160,750	Conf. Fl. Bt. Flat Gr. Bt.
10	10,000,000	6,000	12,000		12	43	23+	110	652	312,500	Conf. Fl. Bt. Granary W.
11	593,000	14,000	20,300		8	10	23+	297 15,400 mites	1,294	1,590,900	Conf. Fl. Bt. Flat Gr. Bt.
12	919,000	510,000			11	18	23+	88	945	539,000	Conf. Fl. Bt. Flat Gr. Bt.
13	62,406		135,000		17	26	23+	194	1,622	820,850	Conf. Fl. Bt. Flat Gr. Bt.
14	40,000	30,000	11,000	1,450	5	28	23+	303	1,811	2,322,000	Conf. Fl. Bt.
18	32,000	<100	14,000	200	3	11	0	0	0	0	
19	267,000	403,000	14,000	1,150	2	10	3	81 19,750 mites	1,111	193,300	Conf. Fl. Bt. Flat Gr. Bt. Granary W.

20	907,000	460,000	49,300	650	1	18	0	3	11	2,050	Conf. Fl. Bt.
											Flat Gr. Bt.
22	112,000	72,000	1,500	80	3	48	20	9	120	6,525	Conf. Fl. Bt.
											Flat Gr. Bt.
23	8,000	14,000	16,000	460	14	15	23+	153	1,356	741,500	Flat Gr. Bt.
24	386,600	349,000	1,000	400	3	4	23+	4	62	1,050	Conf. Fl. Bt.
25	53,300	<100	138,000	1,700	11	14	23+	72	1,534	202,800	Conf. Fl. Bt.
											Flat Gr. Bt.
26	120,000	17,000	102,000	4,100	6	122	23+	15	224	6,200	Conf. Fl. Bt.
											Flat Gr. Bt.
27	500,000	1,000	1,143,000	4,150	30	38	12	5,530	3,931	14,875,000	Flat Gr. Bt.
29	2,700	1,000	152,000	50	3	15	23+	4	94	100	
30	13,300	<100	107,000	200	7	27	20	0	243	1,300	
31	390,000	49,300	61,300	3,000	2	14	20	229	598	16,000	Conf. Fl. Bt.
								3,000 mites			Flat Gr. Bt.
32	43,800,000	31,000,000	426,670	22,650	1	42	20	263	3,110	7,835,000	Conf. Fl. Bt.
											Flat Gr. Bt.
33	272,000	82,670	3,300	250	9	14	23+	600	28	100	Conf. Fl. Bt.
								580 mites			
34	38,600	38,600	2,000	1,650	3	5	0	0	0	0	
35	4,700	6,000	1,300	50	3	4	0	0	24	0	
36	613,300	492,000	4,500	100	10	14	3	0	0	0	
44	211,000	154,670	880	200	17	33	3	1	8	0	
50	6,173,000	6,260,000	42,800	250	6	8	23+	10	61	100	Flat Gr. Bt.

[1] Confused Flour Beetle.
[2] Flat Grain Beetle.

TABLE VII

THE EFFECT OF BLEACHING AGENTS ON THE MICROBIAL CONTENT OF FLOUR. AVERAGE VALUES (ALL MILLS)

Microbial Category	Average No. of Bacteria and Fungi per g.		% of mills showing reduced nos. in bleached flour
	Unbleached Flour	Bleached Flour	
Mesophiles			
Total count (N.A. —37°C.)	59,400	24,000	81
Total count (P.D.A. 22°C.)	28,000	11,000	
Bacterial spores	413	101	66
"Molds"	3,060	1,570	78
Thermophiles			
Total aerobic spores	9.5	11.4	70
"Flat sour" spores	5.0	6.1	62
Anaerobic spores	9.0	9.8	72

since the total data would allow for some 5,000 different combinations. The necessary selection has been influenced by two main purposes:

(1) to explore those categories of measurements between which some biological relationship might be expected; (2) to estimate the effect of specific mill sites in influencing insect and microbial population in flour.

The calculation of Spearman's rank-order correlation coefficient (Kendall, 11) is less cumbersome than some other methods of estimating statistical significance, and furthermore avoids undue "weighting" of results by a few extreme values.

Before adopting this method, it seemed desirable to obtain some indication of its suitability for this particular study. The first calculations were, therefore, made between sets of data which would be expected to have a direct biological interdependence. Thus, since larvae are the most actively defecating phase of mill insects and since the number of whole insects at a particular site might be expected to bear a relation to the numbers of insect-fragments and these in turn to the numbers of microscopic fragments after passing through the mill machinery, these particular relationships were first tested. As indicated in Table VIII the rank-order correlation coefficients for these sets of data were as follows: larvae from boots (site 5) vs. excreta from boots (site 5) — 0.86; insects (site 5) vs. fragments (site 5) — 0.77; total insect fragments (sites 2 - 7) vs. microscopic insect fragment count of

finished flour 0.65. All these values are well above the coefficient of 0.42 which is required to indicate that a correlation could occur by chance alone with a 1% probability. These results were considered to indicate the suitability of the method of calculation and the adequacy of the technical methods used in obtaining the data.

The selected pairs of data and their correlation coefficients are listed in Table VIII. Fig. 1 presents graphically the data between which significant correlations have been calculated. All correlation coefficients were deduced from the total data of all mills; not only the selected mills of the "superior" and "sub-standard" categories.

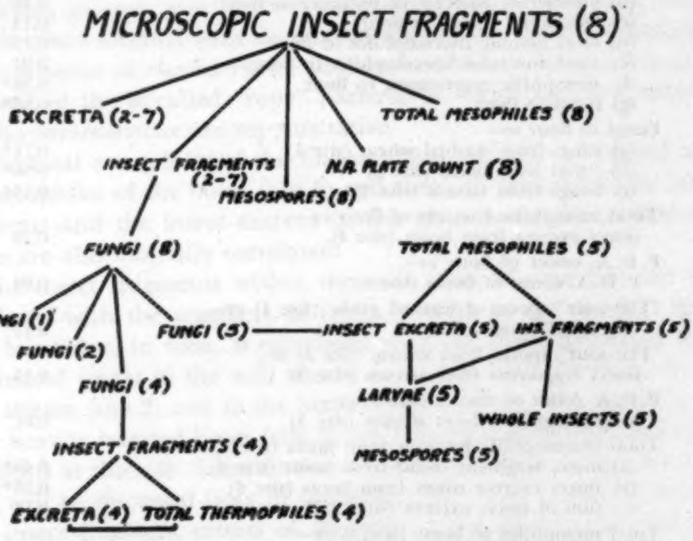

Fig. 1. A diagram of computed correlations between insect matter and microorganisms in a representative group of Canadian Flour Mills. Categories joined by lines represent groups of data from all the mills studied between which a significant correlation has been calculated by the rank-order method. (1) whole wheat from stream; (2) Flour stock (early in the stream); (3) Flour stock (later in the stream); (4) low grade and tailings boots (composite sample); (5) middlings boot; (6) Bran; (7) Shorts; (8) Finished flour.

Figure 1 shows that the microscopic insect-fragment count of the finished flour (specimen No. 8) is correlated: (1) with the sum of the insect-fragment counts for the sites 2 to 7 within the mill, (2) with the sum of the insect excreta from sites 2 to 7, (3) with the plate count (drop-plate method; 37°C. 48 hours) and (4) with the total mesophilic bacterial count (nutrient agar count at 37°C. plus potato-dextrose agar count at 22°C.)

TABLE VIII

CORRELATIONS BETWEEN SETS OF INSECT AND MICROBIAL DATA OBTAINED FROM 32 FLOUR MILLS

DATA COMPARED	COEFFICIENT VALUE
Microscopic insect fragment count of finished flour (site 8) *vs*—	
(a) total insect fragments in the mill (sites 2–7)	0.65**
(b) nutrient agar plate count of flour	0.34*
(c) total mesophilic bacterial count of flour	0.31*
(d) sum of insect excreta counts (sites 2–7)	0.65**
(e) mesophilic spore count in flour	0.35*
Sum of insect fragments from the mill sites 2–7 *vs*—	
(a) mesophilic bacteria of bleached flour	0.18
(b) mesophilic bacteria of unbleached flour	0.16
(c) "flat-sour" spores in flour	0.13
(d) total aerobic thermophiles in flour	0.17
(e) total anaerobic thermophiles in flour	0.01
(f) mesophilic spore-count in flour	0.35*
(g) fungi in flour	0.36*
Fungi in flour *vs*—	
(a) fungi from washed wheat (site 1)	0.71**
(b) fungi from boots (site 5)	0.36*
(c) fungi from stream (site 2)	0.55**
Total mesophilic bacteria of flour *vs*—	
insect excreta from boots (site 4)	0.26
P. D. A. count of flour *vs*—	
P. D. A. count of boots (site 5)	0.04
"Flat-sour" spores of washed grain (site 1) *vs*—	
"flat-sour" spores of flour	0.17
"Flat-sour" spores from stream (site 3) *vs*—	
insect fragments from stream (site 3)	0.15
P. D. A. count of stream (site 3) *vs*—	
insect fragments from stream (site 3)	0.01
Total thermophilic bacteria from boots (site 4) *vs*—	
(a) insect fragment count from boots (site 4)	0.49**
(b) insect excreta count from boots (site 4)	0.58**
(c) sum of insect excreta (sites 2–7)	0.13
Total mesophiles of boots (site 4) *vs*—	
whole insects from boots (site 4)	0.13
Fungi from boots (site 4) *vs*—	
insect fragments from boots (site 4)	0.50**
P. D. A. count from boots (site 4) *vs*—	
insect fragments from boots (site 4)	0.15
Total mesophilic bacteria from boots (site 5) *vs*—	
(a) insect fragment count from boots (site 5)	0.55**
(b) insect excreta count from boots (site 5)	0.55**
Larvae from boots (site 5) *vs*—	
(a) insect excreta from boots (site 5)	0.86**
(b) mesophilic spores from boots (site 5)	0.61**
Whole insects from boots (site 5) *vs*—	
insect fragments from boots (site 5)	0.77**
Fungi from boots (site 5) *vs*—	
insect excreta from boots (site 5)	0.56**

* Significant at the 5% level.
** Significant at the 1% level.

The sum of insect-fragment counts within the mill (sites 2 - 7) is not only correlated with the sum of insect excreta counts from the same sites, but is also correlated with the mesophilic spore count in the finished flour. A high proportion of the *Bacillus* colonies included in this latter count and isolated during the early phases of this study were rope-forming strains of *Bacillus mesentericus.*

The positive determination of potential "rope" formers among the *Bacillus* strains recovered was too time-consuming to consider for the thousands of *Bacillus* colonies encountered. However, a large proportion of the numerically dominant mesophilic spore-forming rods recovered by this method provided highly viscous colonies on media containing glucose, and in the young state, would form long elastic threads when touched with an inoculating needle. This manifestation of the presence of elastic, viscid, polysaccharide capsular gums is characteristic of the so-called "rope" bacteria of which certain strains of *Bacillus mesentericus* are representative.

The total mesophiles of the finished flour are also correlated with the mesophiles of the boots (site 5) and these, in turn, with the insect fragments and the insect excreta in the same boots. These last two values are also mutually correlated.

The insect fragments within the mill (sum of sites 2-7) are also correlated with the numbers of fungi (molds) in the finished flour. This last value, in turn, is correlated with the fungi recovered from the washed wheat in the mill stream as well as with the fungi in the flour stream (site 2) and in the boots (sites 4 and 5). The fungi in the more heavily infested boots (site 4) are also correlated with the insect fragments at this site. Some further evidence for these fungal relationships will be discussed later in the section on "microscopic evidence". The insect fragment counts of the same boots (site 4), in addition, are correlated with the total thermophiles at this site, though a correlation was not established between thermophiles of the boot and in the finished flour.

It should be noted in Table VIII that the sum of insect fragments from the intermedial mill sites is *not* significantly correlated with the total mesophilic bacteria in flour. This might seem to be a contradiction to the significant correlation established between insect fragments in the mill and the microscopic fragments in the flour, this latter value in turn being correlated with the mesophilic bacteria in the flour. On reflection, however, it may be seen that these relationships would appear to be valid, since much of the total "large" fragments estimated in the sum from intermediary sites are mechanically

removed and to a different degree depending on mill equipment. A fraction of them become further subdivided, and these, with those fragments already small enough to avoid mechanical rejection, constitute the "microscopic" fragment category of the finished flour. These are the fragments whose *numbers* are correlated with bacterial counts.

The positive relationship between excreta and bacterial numbers (Table VIII) would seem to be valid since concentrated preparations of insect excreta obtained by appropriate sieving from specimens derived from the more severely infested mills and shaken (not crushed nor comminuted) in the manner used for preparation of flour dilutions provided high bacterial and fungal counts as listed in Table IX.

Living and dead insects were shaken with sterile water in the same manner. Their contribution to the microbial count is summarized in Table X. For the most part, fewer microorganisms were recovered from living insects than dead insects, the latter being variable in effect, the number contributed possibly being dependent upon their state of decay. The living flat-grain-beetles and confused-flour-beetles seemed to contribute relatively few bacteria, which might suggest their ability to secrete antibacterial substances. This possibility has been proposed elsewhere.

TABLE IX

THE NUMBERS OF BACTERIA AND FUNGI WASHED[1] FROM INSECT EXCRETA PELLETS OBTAINED FROM "DEAD" STOCK IN FLOUR MILLS

	Numbers per Gram of Seived Excreta Concentrates			
Mill No.	N.A. Plate Count	P.D.A. Plate Count	Meso. Bact. Spore Count	Fungi
3	1,100,000	800,000	43,000	106,000
32	50,000,000	10,000,000	40,000	560,000
27	51,000,000	10,000,000	2,060,000	1,300,000
11	2,100,000	13,000	1,400,000	20,000
14	10,000,000	20,000	1,300,000	10,000

[1] "Washed" by the procedure used to obtain serial dilutions of flour: excreta not disintegrated.

Median Values: Insect and Microbial Categories—All Mills. A secondary objective in this study was to be able to estimate the median standard of mill sanitation currently being practiced. To this end, the median values for all categories of insect and of microbial data have been determined, and are presented in Tables XI and XII, respectively. The median values are frequently much greater than those

TABLE X

THE AVERAGE NUMBER OF BACTERIA AND FUNGI RECOVERABLE FROM MILL INSECTS BY WASHING

AVERAGE NUMBER PER INSECT

Insect Species	Living Insects				Dead Insects			
	N. A. Count 37°C.	P. D. A. Count 22°C.	Meso-philic Spores	Fungi	N. A. Count 37°C.	P. D. A. Count 22°C.	Meso-philic Spores	Fungi
Confused flour beetle	2,300	<100	970	70	18,000	34,000	40	700,000
Flat grain beetle	<100	<100	1,600	260	900,000	<100	50	17,000
Black flour beetle[1] (*Tenebrio*)	760,000	180,000	3,000	140,000	17,000	1,600	1,300	5,000
Mediterranean flour moth larvae	not counted	not counted			2,000,000	1,500,000	300	46,000

[1] Note: This beetle is very much larger than the others tested.

representative of "superior" mills (Tables I - IV), which establishes that a number of mills are severely lax in their attempts to control insect infestation.

TABLE XI

MEDIAN VALUES AND THE ARITHMETIC MEANS (ALL MILLS) OF VARIOUS INSECT CATEGORIES AT SELECTED MILL SITES

Insect Material		Mill Sites							
		1	2	3	4	5	6	7	9
Adult insects	M.V.[1]	0	0	0	10	14	0	0	0
(per 100 g.)	A.M.[2]	0	0	3.7	1,257	447	0	0.1	0
Fragments	M.V.	0	2	10	234	307	0	0	0
(per 100 g.)	A.M.	0	9.1	21.5	704	815	3.0	0.7	0
Larvae	M.V.	0	0	0	10	16	0	0	0
(per 100 g.)	A.M.	0	125	3.7	21.1	989	0	0	0
Excreta	M.V.	0	0	0	16,000	37,100	0	0	0
(per 100 g.)	A.M.	0	203	540	950,134	1,227,480	0	0	0
Eggs	M.V.	0	0	0	0	0	0	0	0
(per 100 g.)	A.M.	0	0	0	0	0	27.3	7.4	0
Microscopic insect fragments	M.V.	..	..	..	..	..	..	..	904
(per 50 g.)	A.M.	..	..	..	..	..	..	..	3,542

[1] M. V. = Median Value.
[2] A. M. = Arithmetic Mean.

It is interesting to note the trend in bacterial, fungal and insect values as revealed by the median values for the respective mill sites. A marked reduction occurs as the endosperm is separated from the outer seed coats of the grain. Microbial numbers progressively increase from that point onwards.

Numerically Dominant Microbial Species. Table XIII lists numerically dominant mesophilic bacteria and fungi isolated from various mill sites, from specific mill insects and from excreta separated from the "flour" of infested boots. The species named are those which appeared most consistently on plates of the several media at the highest dilutions that provided an acceptable count. A wide variety of microorganisms was observed, and, as might be expected, common omnipresent types were among them. However, there is some indication that the conditions prevailing in severely infested boots do favor the introduction of species not dominantly present on the grain but which may be recovered from insects and insect feces. For instance, many of the *Penicillium* spp. in the flour, in the opinion of an expert mycologist, were not those common to grain, while it may be seen that bacteria not among those dominant on grain may be recovered from

TABLE XII

MEDIAN VALUES (FROM 32 MILLS) OF VARIOUS MICROBIAL CATEGORIES AT SELECTED MILL SITES. NUMBERS PER GRAM

Microbial	Mill Site							
	1	2	3	4	5	6	7	9[1]
Mesophiles								
Bacterial count N. A. 37°C.	74,000	6,200	12,000	88,000	123,000	281,650	382,000	7,850
Bacterial count P. D. A. 22°C.	78,000	10,000	4,000	48,600	61,670	230,000	372,000	3,700
Bacterial-spores				650	150			200
Fungi	400	250	440	18,000	14,000	1,200	4,650	650
Thermophiles								
Aerobic spores	2.5	3.8	4.5	14.4	18.1	12.0	12.5	11.9
"Flat-sour" spores	0.5	2.5	2.5	5.0	6.3	2.0	3.2	3.0
Anaerobic spores	6.4	11.5	20.0	20.0	11.5	0	2.8	6.4

[1] Bleached.

TABLE XIII

THE NUMERICALLY DOMINANT[1] MICROORGANISMS ISOLATED FROM VARIOUS MILL SOURCES

MILL INSECTS					MILL PRODUCTS			
Confused Flour Beetle	Medit. Moth	Medit. Moth Larvae	Flat Grain Beetle	Yellow Meal Worm	Grain from Stream	"Flour" from Boots	Excreta from Boots	Finished Flour
FUNGI								
Aspergillus flavus-oryzae	Mucor-racemosus	Mucor-racemosus	Penicillium spp.	Penicillium spp.	Aspergillus flavus-oryzae	Aspergillus flavus-oryzae	Aspergillus flavus-oryzae	Penicillium spp.
Penicillium spp.	Penicillium spp.			Syncephalastrum racemosum	Penicillium spp.	Penicillium spp.	Penicillium spp.	Aspergillus flavus-oryzae
Cylindrium spp.	Aspergillus versicolor				Mucor racemosus	Mucor racemosus		Aspergillus glaucus
Actinomyces sp. 1						Aspergillus glaucus		
						Scopulariopsis brevicaule		
						Actinomyces sp. 1		
BACTERIA								
Sarcina lutea	Bacillus mesentericus	Bacillus (rope)	Micrococcus spp.	Bacillus spp.	Bacillus spp.	Bacillus spp.	Bacillus mesentericus	Flavobacterium spp.
Sarcina flava	Bacillus (rope)				Achromobacter	Bacillus (rope)	Bacillus (rope)	Bacillus spp.
Achromobacter	Pseudomonas sp.		Bacillus (rope)		Pseudomonas	Flavobacterium spp.	Achromobacter spp.	Bacillus (rope)
	Alcaligenes fecalis	Achromobacter sp.			Micrococcus spp.	Achromobacter spp.	Pseudomonas spp.	Achromobacter
					Flavobacterium	Serratia	Serratia spp.	Micrococcus candidus
						Micrococcus spp.	Flavobacterium spp.	Alcaligenes fecalis
						Yeasts	Sarcina flava	Serratia spp.
							Micrococcus candidus	
							Yeasts	

[1] Species which appeared most consistently on plates of the various media poured at the higher dilutions.

specific insects, from feces and also from flour, (e.g. *Serratia* and *Alcaligenes*). Table XIII, in conjunction with Table XII, provides some basis for the suggestion that "rope-forming" *Bacillus* spp. are contributed from insects and feces in dirty boots.

Microscopic Evidence. The photomicrographs, arranged in Figs. 2-4 record some of the typical microscopic insect fragments recovered from finished flour by the new method described below. The photographs represent some of the larger fragments recorded in this category. Wing-scales from the Mediterranean moth, the pectinate setae, legs and mouth parts of mites, mite larvae (0.1 to 0.3 mm. in length) fragments of exoskeleton of confused flour beetle, mouth parts, appendages and terminal abdominal segments of larvae could be recognized. Each of these fragments was counted as a unit. Preliminary experiments using insects thoroughly ground up in the laboratory indicated that up to 100 microscopic fragments could be recovered from each whole insect, depending on the size of the insect.

The degree of contamination of the contents of boots of certain mills is indicated by the photographs of specimens of insect excreta shown in Fig. 2 (1-2). Feces from four types of insect are apparent in a single specimen.

Reference has already been made to several correlations between fungi at different sites in the mill. Microscopic evidence to lend some support to these relationships is provided by Fig. 3 (1-3). The photographs were taken at a magnification of about 1400× and reveal mycelium in the microscopic fragments of wheat pericarp (bran) which find their way into the flour. The fungus cells are not always the *pseudo-sclerenchymatous* aggregates of the *Dematicaeae* as described by Christensen (5), but some may be hyaline mycelia that are better seen by the Galileo phase-contrast microscope than by direct illumination. These photographs were prepared with the Galileo instrument, with which it is possible to cause the "background" of flour tissue to recede in apparent intensity relative to the fungus cells. The photomicrograph Fig. 3 (6) shows the decayed adbomen of a confused flour beetle larva filled with what appear to be fungus spores. Several such specimens were seen. Preparations of dead insects frequently gave rise to suspensions of fungus spores as may be seen in Fig. 3 (5) (photographed with phase-contrast equipment). Thus it would seem reasonable to suggest that the wheat pericarp contributes part of the total fungal complement of the flour, but that mill conditions which allow for the establishment of relatively large numbers of insects in "dead" stock seems also to be a contributory factor.

Fig. 2. Insect fragments recovered from flour by the flotation method. The long rod-like structures are wheat trichomes (hairs from the "beard" and are included to indicate comparative size. (1) Fragments of mites and mite larva (141x); (2) the inner facet of the head of a confused flour beetle larva and the terminal segment of the antenna of the same species (141x); (3) *Tribolium* antenna with several fragments of mites (141x); (4) and (5) *Tribolium* heads (141x); (6) a crowded field showing *Cadelle* larva (a), mite fragments (b), appendages of unknown insects (c), and exoskeleton fragments (35x) (d); (7) *Tribolium* mandible (71x); (8) fragment of translucent larval skin showing circular seta opening, and a fragment of exoskeleton (141x); (9) abdomen of *Tribolium* larva (?) and part of thoracic segment (71x); (10) terminal part of insect leg (141x); (11) thorax and leg of *Tribolium* (71x); (12) fragments of exoskeleton of adult yellow meal worm *(Tenebrio)* (71x); (13) leg of a mite (141x); (14) a young *Cadelle* larva (35x); (15) a fragment of wheat epidermis and small fragments of exoskeleton (141x).

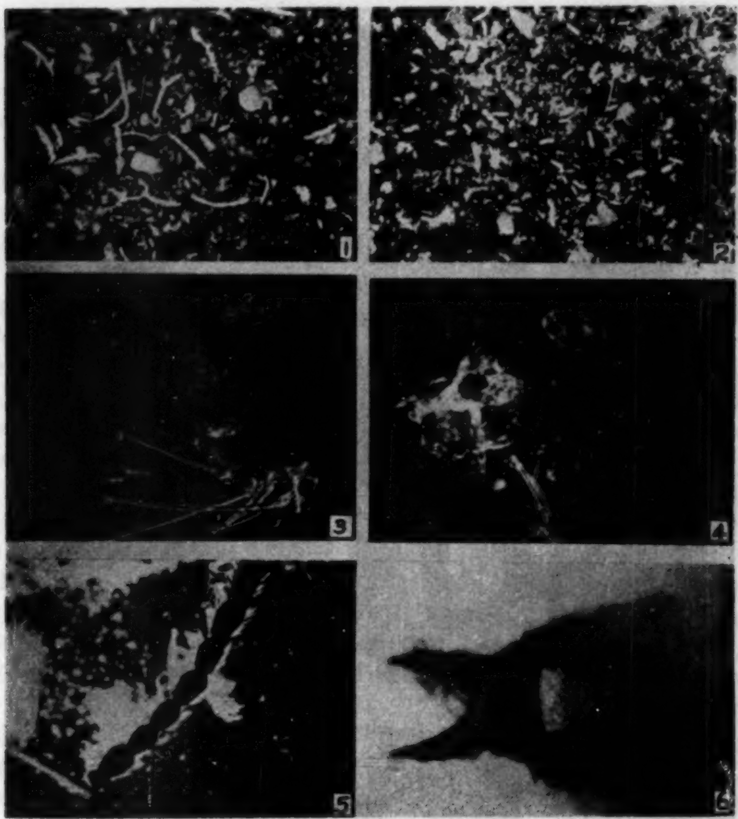

Fig. 3. (1) and (2) insect excreta from infested boots, including feces from yellow meal worm, confused flour beetle, Mediterranean flour moth larvae and an unrecognized specimen (10x approx.); (3), (4) and (5) mites and sundry insect fragments as seen under the dark field provided by the Galileo phase-contrast equipment. Note the fungal spores surrounding a decaying insect fragment (130x); (6) abdomen of a *Tribolium* larva apparently filled with fungal spores (130x).

Discussion and Conclusions

Any conclusions deduced from this study are subject to the criticism that the data refer only to a single inspection of each mill. Clearly the actual infestation reported for a particular mill will be influenced by such factors as the interval between inspection and fumigation or "clean up" action; the proximity to a "shut-down" period, the season of the year, etc. However, while the average relative cleanliness of a particular mill may not be determined from a single inspection, nevertheless, a reasonable estimate may be made of the median

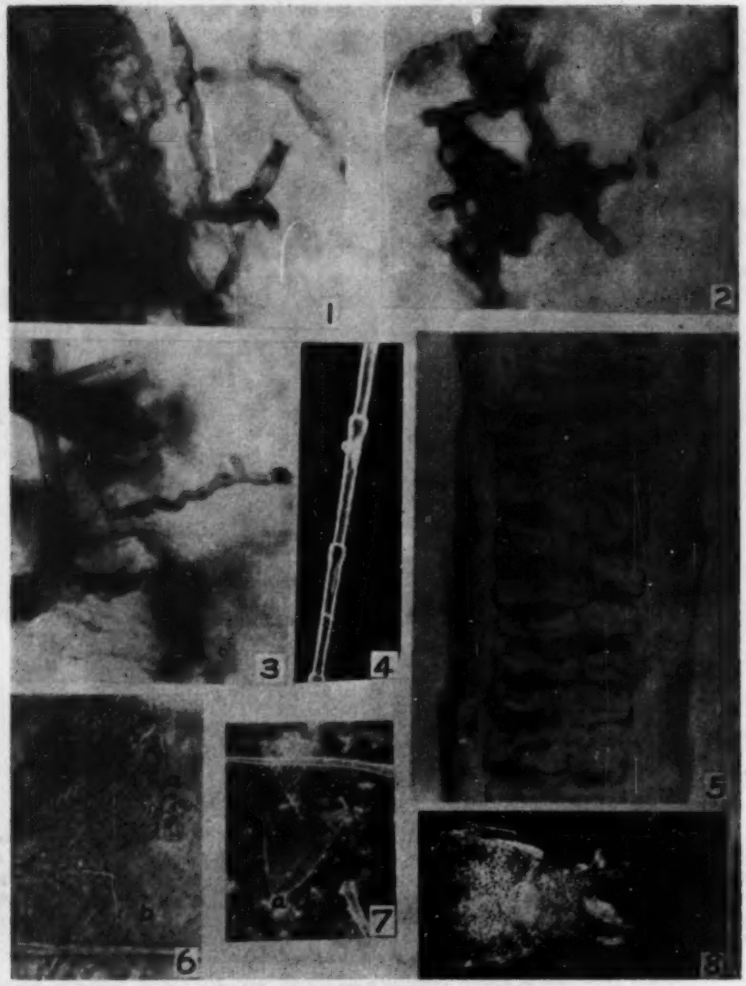

Fig. 4. (1), (3) Fungus cells embedded in wheat pericarp tissue (recovered by flotation); detection improved by phase-contrast microscopy (621x approx.); (4) feather barbule from flour (phase-contrast) (177x); (5) rat-hair recovered from flour showing characteristic internal arrangement of pigment-granules (1332x); (6) and (7) larval skin (a), compared with plant epidermis (b) (177x); note cell walls with characteristic pits in (b); (8) decaying larval fragment filled with fungus spores (177x).

condition of the mills as a group, from which appropriate future action may be deduced.

Since completing this report the study by Harris *et al.* (10) of the relation between insect infestation of wheat and the presence of insect

fragments in flour from U.S. mills has been published. The present Canadian study may be considered to complement the U.S. study in that emphasis is directed to mill sanitation and its effect upon insects and microorganisms in flour.

A new method for estimating microscopic insect fragments in flour shows promise as an indicator of mill sanitation since it is shown to be correlated with total insect fragments found at intervals in the mill (sum of sites 2 - 7) and with insect excreta counts.

The method requires careful familiarization with insect and plant fragments. Its potential value for routine plant control is questionable but seems to be of positive inspectoral value in the hands of experienced microscopists.

Before placing undue confidence in indications of correlation between insect fragments and plate counts of mesophilic bacteria, another survey will be undertaken. A significant correlation, while providing evidence of a numerical relationship between data, does not necessarily indicate interdependent biological relationship. Thus, while a number of categories of insect material are shown to be correlated with various microbial categories, a direct biological relationship need not be assumed. On the other hand, it is entirely likely that those conditions of mill operation which permit the multiplication of insects and the accumulation of insect webbing and excreta would most likely be those which would provide for accumulation of bacteria-laden dust as well as providing for bacterial multiplication by provision of moisture from insect feces and dead insects as well as from crushed insects in the machinery. The humid conditions in "stale", heavily infested boots should be expected to provide extensive bacterial and fungal multiplication with subsequent continuous contamination of elevator carriers and conduits in general. It is quite probable also that accumulation of dirty "dead stock", especially if moistened by contact with damp floors or by insects, would encourage multiplication of thermophilic bacteria with subsequent unsuitability of contaminated flour for the canning industry and to perhaps a lesser degree for bread. Large quantities of bread spoiled by anaerobic thermophiles have been brought to the attention of the senior author. It is interesting to note that the total thermophilic bacteria in boots (site 4) show highly significant correlation with the insect fragment count and the insect excreta count in the same boots. Further study is intended on the thermophile problem in flour.

Acknowledgments

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STUDIES ON THE RELATION OF THE PENTOSANS EXTRACTED BY MILD ACID TREATMENTS TO MILLING PROPERTIES OF PACIFIC NORTHWEST WHEAT VARIETIES[1,2]

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ABSTRACT

The physical properties of water-soluble flour pentosan preparations and the possibility that endosperm-cell-wall properties are related to milling behavior suggested that an empirical procedure might be developed, useful for predicting the milling acceptability of Pacific Northwest soft wheats.

After extraction with 80% ethanol to remove free sugars and levosin, milling fractions (flour, shorts, and bran) from four wheats differing in milling behavior were extracted with hydrochloric acid under conditions that avoided dispersion or degradation of starch. The amounts of pentosans removed were determined (as pentose) by an orcinol procedure. Flours from the four wheats differed little in extractable pentosan content, and yielded the least pentosan of the three milling fractions. The shorts samples, which showed the largest relative differences in yield between the wheats, contained more than three times the extractable pentosan in the flours. By varying the severity of acid treatment, indications were obtained that the bran coats were not the principal source of the pentosans extracted by the milder acid treatments

Wheat samples (ground to pass 40-mesh screen) were extracted with 2.0 *N* hydrochloric acid for 6 hours at 25°C. With a series of 39 samples, including ten varieties grown at four locations in 1950, the pentosan extracted (2.31% to 3.34%) was correlated with both a total milling score and straight flour yield obtained on a Buhler experimental mill ($r = -0.70^{**}$ and -0.75^{**}, respectively). Test-weight, total-nitrogen, and crude-fiber data did not give significant correlations with either milling score or flour yield.

In earlier attempts to account for variations in milling behavior of wheats, physical methods in general, including microscopic examination of the structure of kernels and milled products (3, 7, 10, 11), have been used predominantly. Efforts to relate compositional differences to milling behavior seldom have been reported, although they could be expected to accompany structural or other physical differences.

The hemicelluloses appeared to be a class of constituents that might influence milling acceptability. This possibility was suggested by observations of the physical properties of water-soluble flour pentosans (12). The preparations appeared fibrous and were difficult to grind;

[1] Manuscript received July 28, 1952. Presented at meeting of Pacific Northwest Section, American Association of Cereal Chemists, Great Falls, Montana, June, 1952.

[2] Contribution from Western Regional Research Laboratory, Albany, Calif., Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

and increasing difficulty in grinding with increasing moisture content recalled the necessity, in commercial practice, of tempering certain poor-milling varieties, e.g., Rex, only to rather low moisture levels, around 12.5%. The report of Greer and Hinton (6) on the manner of fracture of endosperm particles suggested further that the nature of endosperm cell walls might influence milling behavior; and such cell-wall material could be expected to contain appreciable proportions of pentosan materials. Also the suggestion was made[3] that a concentration of endosperm-cell-wall material into the "white" or "fuzzy" shorts occurs in milling; and the observations reported by Hay (8) appeared to support the suggestion.

More recently Wolf, Seckinger, Rosewall, MacMasters, and Rist (18) and Larkin, MacMasters, and Rist (10) have shown, with samples of seven varieties grown in the Pacific Northwest, that endosperm cell wall separated from these wheats consisted of from 20 to 50% of pentosan material; that differences in endosperm-cell-wall thickness occurred between samples; and that in general as the thickness of cell wall increased, difficulties in milling increased.

The object of the research reported below was to study the relationship of milling acceptability of Pacific Northwest soft wheats to the nature or amount of pentosan or other hemicellulose constituents in the wheats. This paper is concerned with the pentosans extractable by mild acid treatment. The development of an empirical, relatively simple, procedure for extraction and determination of pentosans is described, together with its application to certain milling fractions as well as to a series of thirty-nine wheat samples of the 1950 crop which differed in milling quality.

Materials and Methods

Samples were obtained through the cooperation of the Western Wheat Quality Laboratory, U. S. Bureau of Plant Industry, Soils, and Agricultural Engineering, at Pullman, Washington. All were individual samples from one-fortieth-acre-plot trials. The Western Wheat Quality Laboratory also supplied test weight and milling data. Determination of weight per bushel was made by the method prescribed by the Official Grain Standards of the United States (1); moisture contents of the samples when test weight was measured were near 10%.

Test milling was carried out on a Buhler experimental mill. Details of the procedures have been published (15). The milling score is calculated from straight and patent flour yields, flour ash content,

[3] Private communication from E. F. Seeborg.

tempering moisture, and milling time, by the method described by Seeborg (16).

Analytical methods for total nitrogen, crude fiber, and moisture (air oven, 135°C., 2 hours) were those of the Association of Official Agricultural Chemists (2).

Extraction and Determination of Pentosans

In exploratory work, the amounts of pentosan that could be extracted from whole wheat and flour samples by water and dilute salt, acid (hydrochloric and sulfuric), and sodium hydroxide solutions were determined, with extraction times and temperatures that avoided dispersion or degradation of starch. Mild hydrochloric acid treatments appeared most likely to be useful, because they removed roughly twice as much pentosan from flour as extraction with water or dilute salt solutions; and the amounts obtained in acid extracts of whole wheats gave indications of reflecting milling-behavior differences while the amounts of water-extractable pentosans did not. The use of alkali had no advantage because, as alkali concentration was increased, dispersion of starch became marked before extraction of pentosan increased appreciably. Using hydrochloric acid, a limited survey then was made of the effects of varying acid concentration, time, and temperature, and three sets of extraction conditions were selected for use in further work. These were (A) 0.5 *N* hydrochloric acid, 0.5 hours, 50°C.; (B) 2.0 *N* hydrochloric acid, 6 hours, 25°C.; and (C) 2.0 *N* hydrochloric acid, 24 hours, 25°C. The basis for choice of these extraction conditions will be presented later in a section dealing with milling fractions.

Extraction Procedures. The procedures were as follows, using conditions (B) for illustration. Samples were ground to pass a 40-mesh screen in a small Wiley mill. One-hundred-mg. portions were placed in conical centrifuge tubes of 12-ml. capacity and 10 ml. of 80% aqueous ethanol was added to each tube. The tubes were placed in a water bath at 75° ± 5°C., and the contents dispersed occasionally by shaking. After 10–15 minutes, they were centrifuged and the supernatant liquids carefully decanted. Three additional extractions with 80% ethanol were made in the same manner and the alcoholic extracts, containing free sugars, were discarded. Ten ml. of 2.0 *N* hydrochloric acid then were added to each tube. (After the final 80% ethanol extraction, free liquid was carefully drained from the tubes, but the residue was not dried. The adhering alcohol facilitated dispersion of the residue in acid; no correction was made for its small

effect on the acid extract volume.) The tubes were stoppered, shaken vigorously to disperse the samples, and rotated end-over-end for 6 hours while immersed in a water bath held at 25°. After centrifuging, 5-ml. portions of the supernatants were diluted with water to 50 ml.

Reproducibility of results was improved by filtering the diluted extracts through No. 1 Whatman paper. However, filtration removed some pentosan, and decreased slightly the range of values found. The use of filter paper also made necessary a small blank correction in the pentose determination. In filtering, the folded paper was filled with diluted extract, allowed to empty completely, and this first portion of filtrate (about 20 ml.) discarded; but this procedure did not eliminate entirely the material responsible for the blank.

Estimation of Pentosan. The pentosan content of the extracts was measured by an orcinol procedure adapted from the methods described by Brown (4) and Drury (5). No hydrolyzing treatment prior to the determination was found necessary.

Reagents: Stock iron solution, 0.990 g. ferric ammonium sulfate 12 H_2O (clear crystalline material was selected) dissolved in one liter of concentrated hydrochloric acid.

Color reagent, 333 ml. stock iron solution, 467 ml. concentrated hydrochloric acid, 200 ml. water, 2 g. orcinol (recrystallized from benzene). The orcinol was dissolved in a portion of the water and added to the acid reagents.

Standard solution, D-xylose, 20 γ per ml. (preserved with sodium benzoate).

Procedure: Aliquots of the standard solution and of the diluted extracts containing 20–40 γ pentoses were placed in test tubes (18 mm. o.d.) and water was added if necessary to give a volume of 3 ml. Nine milliliters of color reagent were added and mixed thoroughly with the sample. The test tubes then were placed in a boiling-water bath (water level at least as high as the level of tube contents) for 25 minutes, removed and cooled immediately in cold tap water. Samples were read against a reagent blank in a suitable photoelectric colorimeter, with light of 670 mμ wave-length. Results of determinations on at least two separate extracts, usually agreeing within 0.1% (expressed as percent of sample), were averaged to obtain the values reported.

Interference by Hexoses. Extraction with 80% ethanol was employed to remove free sugars and levosin (9, 17) from the samples, to minimize interference by hexoses in the orcinol determination of pentoses. Both Brown (4) and Drury (5) have used transmittancy readings taken near 520 mμ to correct the apparent pentose (as determined at 670 mμ) for the contribution of hexoses, especially glu-

cose. When applied to the acid extracts of 80% ethanol-extracted whole wheat, these "dichromatic" methods indicated the presence of only small amounts of hexose, and the amounts were consistently proportional to the amounts of pentose found. Accordingly, such hexose would have no influence on the correlation between apparent pentose and measurements of milling behavior.

Comparison of Milling Fractions

Differences in the milling acceptability of wheats are reflected in the yield of various milling fractions and in the degree to which these fractions represent a separation and concentration of different parts of the kernels. Consequently, it was thought comparisons of the amounts of pentosans extracted from some milling fractions might be useful in selecting conditions for use with whole wheats.

Accordingly, flour, bran, and shorts from millings on a Buhler experimental mill were examined; these fractions were obtained from four wheats differing in milling behavior. Acid extractions were made under the three sets of conditions described above; these conditions

TABLE I

AMOUNTS OF PENTOSANS EXTRACTED FROM MILLING FRACTIONS BY ACID TREATMENTS OF DIFFERING SEVERITY (1950 CROP, PULLMAN SERIES, WHEATS)

Fraction	Variety [1]	Milling Yield	Pentosan Extraction Procedure [2]		
			A	B	C
		%	%	%	%
Shorts	Rex	7.7	4.3	6.5	5.7
	Brevor	5.9	4.0	7.8	7.0
	Golden	4.8	3.5	6.5	7.6
	Elgin	2.9	3.1	7.0	7.4
Flour	Rex	69.2	1.5	1.9	1.4
	Brevor	71.7	1.5	2.0	1.4
	Golden	71.0	1.6	2.0	1.6
	Elgin	77.9	1.3	1.6	1.3
Bran	Rex	23.1	3.6	6.7	8.9
	Brevor	22.4	3.1	7.2	10.2
	Golden	24.2	3.2	6.5	10.0
	Elgin	19.2	2.5	6.8	10.5
Shorts through 8XX–on 10XX	Rex	2.6	4.5	5.5	4.5
	Brevor	2.3	3.9	5.0	4.2
	Golden	1.4	3.5	5.2	4.7
	Elgin	0.7	3.3	5.2	5.0

[1] Milling scores (16); Rex, 76.4; Brevor, 82.5; Golden, 83.0; Elgin, 94.2.
[2] A: 0.5 N hydrochloric acid, 50°C., 0.5 hour; B: 2.0 N hydrochloric acid, 25°C., 6 hours; C: 2.0 N hydrochloric acid, 25°C., 24 hours. All samples extracted with 80% ethanol prior to acid extraction; the diluted acid extracts were not filtered. Results are on a moisture-free basis.

differ in severity of the acid treatment, increasing in severity from A through C. Descriptions of the milling fractions and pentosan extraction data are given in Table I.

The milling fractions differed in their response to changes in acid treatment. All four bran samples showed rather large increases in pentosan extracted from A through C; all four flour samples gave a moderate increase from A to B followed by a decrease from B to C. Under the mildest extraction conditions, more pentosan was extracted from shorts than from bran samples from the same wheat; with the most severe acid treatment, the reverse was found.

From these data, the pentosan extracted from the amount of each fraction representing 100 g. of wheat (i.e., yield × percent extracted pentosan) was calculated (Fig. 1). Under extraction conditions A and B, the total pentosan extracted reflects milling score or flour yield

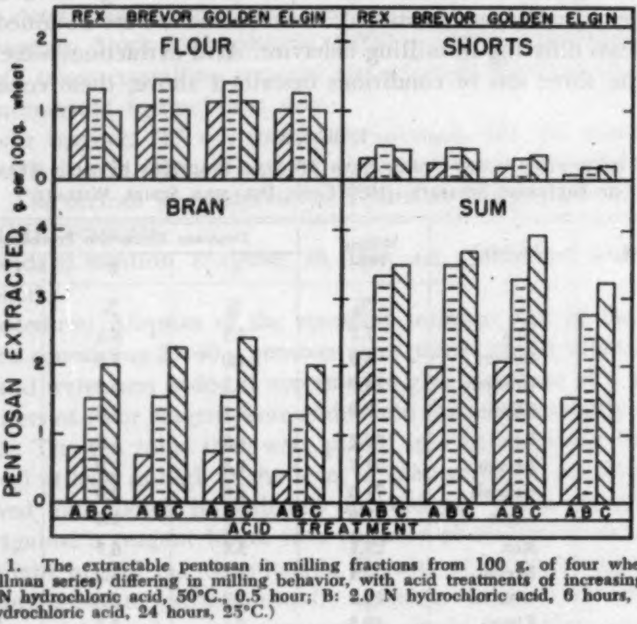

Fig. 1. The extractable pentosan in milling fractions from 100 g. of four wheats (1950 crop, Pullman series) differing in milling behavior, with acid treatments of increasing severity. (A: 0.5 N hydrochloric acid, 50°C., 0.5 hour; B: 2.0 N hydrochloric acid, 6 hours, 25°C., C: 2.0 N hydrochloric acid, 24 hours, 25°C.)

fairly well, and the bran fractions contribute half or less of the total. With extraction procedure C, the total pentosan extracted does not reflect milling performance, and the bran fractions contribute six-tenths or more of the total. Perhaps a more significant difference between conditions B and C is the actual decrease in the contributions of the flour fractions. Thus extraction conditions which allow endo-

sperm rather than bran to contribute the major portion of extracted pentosans appear to provide for significant correlations with milling measurements.

In connection with the differences in extraction of pentosans from endosperm and bran, some additional observations should be reported. In those cases (Table I) where less pentosan was extracted under C than under B, it was found that if the 6-hours 2 N acid extract was removed from the sample and fresh acid added, further extraction of pentosan did occur; while a precipitate formed in the 6-hour extract (allowed to stand an additional 18 hours) and decreased amounts of pentosan remained in the supernatant. Consequently, it appears that in the period between 6 and 24 hours, extraction of pentosan continued, but at the same time precipitation of pentosan already in solution occurred. In the case of the brans, the former process predominated; in the case of the flours, the latter.

Perlin (13, 14) has shown that the major portion of the water-soluble flour pentosans occur as arabo-xylans from which, upon rather mild acid treatment, arabinose units are removed (as the monosaccharide); and the residual xylans are much less soluble than the original pentosans. His work thus provides an explanation for the decreases observed between conditions B and C with the flour samples. Because the bran samples responded differently, the possibility that bran pentosans are of a distinctly different type is suggested, although accessibility, degree of degradation required before extraction, or other factors might account for the apparently different type of behavior.

Actually all four bran samples contained considerable endosperm material, and the particularly poor "clean-up" of the Rex bran would seem to be responsible for the smaller increase between 6 and 24 hours of treatment obtained with the Rex bran as compared to the other brans.

A similar behavior occurred in the shorts samples. The Rex and Brevor wheat samples gave middlings rather difficult to reduce; consequently the shorts contained an appreciable amount of endosperm material, and more pentosan was extracted in 6 than in 24 hours. The Golden and Elgin shorts were relatively free of endosperm material, and increases between 6 and 24 hours were obtained. The data on the shorts through 8XX- on 10XX- sub-fraction also agree with this interpretation. This sub-fraction was obtained in an attempt to separate from the shorts material that was relatively free of bran and germ fragments. Although the yield of this sub-fraction from the Golden and Elgin samples was low, the sub-fractions from all four wheats showed a decrease in pentosan extracted between 6 and 24 hours, and

the differences between the sub-fractions were smaller than with the original shorts. Thus as the middlings were reduced, apparently a residual fraction of endosperm material with high content of extractable pentosans was resistant to conversion to flour.

Examination of Whole Wheat Samples

On the basis of the comparisons of milling fractions, conditions A and B appeared about equally likely to extract amounts of pentosans showing a relationship to milling acceptability. For routine work, the B conditions were more convenient and were chosen for application to a series of 39 wheat samples from the 1950 crop. These included the varieties Baart, Brevor, Elgin, Elmar, Federation, Golden, Marfed, Idaed, Rex, and Triplet, each grown at Moro and Pendleton, Oregon, and Pullman and Lind, Washington (a Golden sample grown at Moro was not available). "Extracted pentosans", total nitrogen, and crude fiber data were obtained, as well as test weight values; and milling score and straight flour yield data were used as measures of milling acceptability. Correlation coefficients calculated from these data are:

	r
Milling score x extracted pentosan	—0.70**
Milling score x test weight	+0.18
Milling score x total N	—0.16
Milling score x crude fiber	+0.12
Straight flour yield x extracted pentosan	—0.75**
Straight flour yield x test weight	+0.09
Straight flour yield x total N	—0.19
Straight flour yield x crude fiber	—0.02

Scatter diagrams for milling score and straight-flour yield plotted against extracted pentosan are shown in Figs. 2 and 3. In this series of wheats, these two measures of milling behavior are more closely related to the extracted pentosan data than to any of the other data obtained. In view of the low, non-significant correlation coefficients with test weight, emphasis should be placed on the facts that the samples included no hard red wheats, and that the varieties represented may differ in milling properties more than those grown in other parts of the United States. However, the conclusion appears justified that with these wheats test weight did not have a predominant influence on milling behavior. In contrast, the amounts of pentosan extracted provide a much better indication of milling performance.

The crude-fiber data were obtained in an attempt to estimate bran-endosperm ratio by a measurement other than test weight; but the

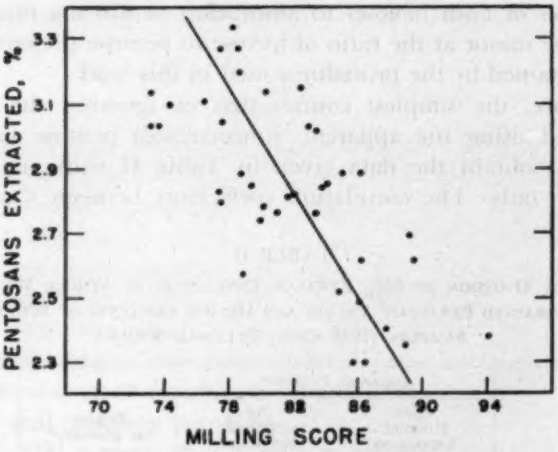

Fig. 2. Scatter diagram showing relationship of milling score to extracted pentosans (whole wheat samples, 1950 crop, representing ten varieties grown at four stations).

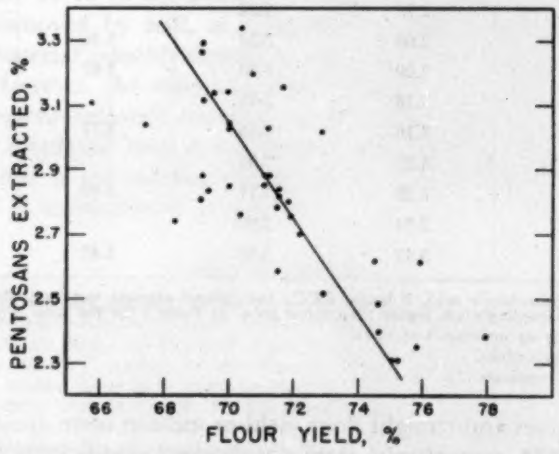

Fig. 3. Scatter diagram showing relationship of flour yield to extracted pentosans (whole wheat samples, 1950 crop, representing ten varieties grown at four stations).

data show no significant relationship between crude fiber content and the milling behavior measurements.

Omission of 80% Ethanol Extraction. If routine use were to be made of a determination similar to the one applied above, omission of the 80% ethanol extraction steps would save much time. The "dichromatic" method of correcting for hexose has been found useful when a single known hexose is present (4), but in aqueous extracts of wheat both glucose and fructose would be present. However, the

contribution of both hexoses to absorption at 670 mμ might be expected to be minor at the ratio of hexose to pentose present in wheat extracts obtained by the procedures used in this work.

Therefore, the simplest course, that of ignoring the effects of hexoses and using the apparent (uncorrected) pentose values, was followed to obtain the data given in Table II with samples from one station only. The correlation coefficients between the apparent

TABLE II

EFFECT OF OMISSION OF 80% ETHANOL EXTRACTION OF WHOLE WHEATS ON EXTRACTED PENTOSAN[1] VALUES AND HEXOSE CONTENTS OF WHEAT SAMPLES (1950 CROP, PULLMAN SERIES)[2]

Variety	Apparent Pentose[3]		Sugars (as glucose)[4]	Fructose[4]
	Prior Ethanol Extraction	No Ethanol Extraction		
	%	%	%	%
Elgin	2.54	2.96	2.93	1.95
Elmar	2.73	3.24	. . .	. . .
Idaed	2.66	3.32	3.34	2.09
Triplet	3.00	3.60	3.07	1.93
Federation	3.18	3.81	. . .	. . .
Golden	3.16	3.54	3.74	2.48
Baart	3.29	3.97	. . .	. . .
Brevor	3.29	3.71	3.88	2.57
Marfed	3.34	3.93	. . .	. . .
Rex	3.42	3.80	3.45	2.15

[1] 2.0 N hydrochloric acid, 6 hours, 25°C.; the diluted extracts were not filtered, and the values given accordingly are higher than those given in Table I for the same wheats.
[2] All values on moisture-free basis.
[3] Orcinol procedure.
[4] A.O.A.C. methods (2).

pentose values and straight flour yield or milling score ($r = -0.85^{**}$ and -0.92^{**}, respectively) were not changed significantly from those obtained using the pentose values after prior 80% ethanol extraction ($r = -0.84^{**}$ and -0.97^{**}).

Considering the six wheats for which values for total sugars (80% ethanol-extractable) are given, the mean value of 3.4% compared to a mean increase of 0.48% in apparent pentose indicates that on the average about 7 units of hexose are equivalent to one of pentose in contributing to the apparent pentose values. Among the six individual wheats, however, a range of 5 to 10 is shown. Even though the hexose contents of the wheats differ and affect the apparent pentose values

in an irregular way, the results suggest further trial of the simplified method.

Discussion

This work was carried out because of the desire expressed by wheat breeders for methods that would evaluate the milling properties of wheats and would be applicable in routine fashion when only small amounts of wheat were available. For such use, a procedure such as described here or one incorporating some of several possible improvements appears to be worth further investigation.

The method as applied to the principal series of samples was arrived at rather empirically. Perlin's studies (13, 14) provided for interpretation of some of the observations; and the information on endosperm cell wall thickness presented recently by Larkin *et al.* (10) and Wolf *et al.* (18) suggests that a reasonable basis exists for use of the method. Their observations and those reported here on milling fractions appear to be in agreement. It may be assumed that the extra pentosan removed by acid, as compared to water, represents hemicellulose material possibly bound to other cell-wall material by ester linkages. However, the extent to which pentosans would be removed from endosperm-cell-wall material by the particular acid extraction conditions employed here is not known, and water-soluble pentosans were included in the extracts. Consequently, further interpretation of the results is unwarranted at present.

Acknowledgments

This work was carried out in informal collaboration with the Western Wheat Quality Laboratory, Bureau of Plant Industry, Soils, and Agricultural Engineering, and the Northern Regional Research Laboratory. We are indebted to Dr. M. A. Barmore and Mr. E. F. Seeborg of the Western Wheat Quality Laboratory for suggesting that studies of milling characteristics be made and for supplying samples and milling data; and to Dr. M. M. MacMasters and co-workers of the Northern Regional Research Laboratory for providing us with information on their studies prior to publication. We also wish to thank Mr. E. F. Potter and Mr. H. M. Wright of this Laboratory for the total sugar, fructose, and crude fiber determinations.

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HEAT DENATURATION OF GLUTEN[1,2]

JAMES W. PENCE, ALI MOHAMMAD, AND DALE K. MECHAM

Abstract

Effects of time, temperature, moisture content, pH, and salt concentration on the denaturation of gluten by heat have been studied. The denaturation of wet, gum gluten was found to be essentially a first-order reaction having an energy of activation of approximately 35,000 calories per mole when measured by a baking-test method and 44,000 calories by a solubility method. Results obtained by the two methods, while differing in magnitude, were found to be parallel in most respects.

The rates of denaturation at both 80° and 90°C. were negligible at low moisture contents but rose rapidly to maxima at 35–40% moisture; at higher moisture levels the rates declined slightly toward intermediate values. Denaturation of wet, gum gluten was slow at pH 4 but became rapid at higher pH values; the relations among pH, temperature, and rate of denaturation were found to be quite complex. At low pH values damage to baking properties occurred which was not caused by heat. Variations in salt concentration had no effect on rate of denaturation by heat, but the presence of salts may influence the damage to baking properties caused by low pH conditions.

Significant variation occurred among the rates of denaturation, as measured by the baking test, of glutens from flours of different quality, but no consistent trend was observed for glutens from flours of better quality to denature at higher or lower rates.

The denaturation of a gliadin preparation was much slower than that of the whole gluten complex and was characterized by a definite induction period.

The alteration of flour proteins by heat has been investigated in the past, principally with regard to heat treatment of flours for improvement of their baking properties or with regard to artificial drying of wheat. Geddes (5, 6) reinvestigated much of the early work and determined the extent of treatments which could be used without damage to baking properties; he concluded, however, that the effect of heat on flour *proteins* was always detrimental to baking quality.

A limited number of observations of the effects of heat on gluten during drying operations have been reviewed by Blish (1). Evidence was found by Sharp and Gortner (14) that the glutenin fraction is far more sensitive to heat than the gliadin portion of gluten. The same conclusion was reached by Cook (2) from viscosity and solubility measurements on gluten dispersions.

The effects of heat on the properties of flour proteins under con-

[1] Manuscript received September 12, 1952.

[2] Contribution from Western Regional Research Laboratory, Albany, Calif. Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

ditions similar to those existing in doughs during the baking process have received little attention, although it could be expected that differences in such effects and in the baking performance of doughs of various types might be related. Because wheat flour is used in such a great variety of baked goods in which it is subjected to a range of pH and moisture conditions and because so little is known regarding the denaturation of gluten, in general, a study was undertaken of the effects of temperature, time of heating, pH, and moisture conditions upon the rate of alteration of gluten properties by heat.

Materials and Methods

For most of the experiments, glutens were obtained from two unbleached, commercial flours containing approximately 10% protein and milled chiefly from blends of Baart and Federation wheats. Other glutens were obtained from unbleached, straight-grade flours experimentally milled from Turkey, Pawnee, and Red Chief wheats grown in Kansas; these flours likewise contained about 10% protein at 14% moisture. A few experiments were conducted with gluten from a flour containing 13% protein and experimentally milled from Mida wheat. Doughs were washed in the pH 6.8, 0.1% phosphate buffer of Dill and Alsberg (3) until the starch was nearly all removed; the gluten was then washed thoroughly in distilled water to remove the buffer salts.

Samples of gluten to be heated (5-6 g.) were sealed in small test-tubes (15x125 mm.) and immersed in a constant-temperature water bath controlled to within ± 0.5°C. After the desired interval of heating, the tubes were transferred to an ice bath to cool as quickly as possible. It was assumed that the time intervals required for temperature equilibration during heating and cooling nearly compensated each other, since results with heating times as short as two minutes were in accord with those obtained for longer heating periods.

Different moisture levels were obtained in some instances by mixing ground, lyophilized gluten in suitable proportions with wet gluten that had been ground in the frozen state with dry ice; after sublimation of the dry ice below 0°C., the samples were allowed to equilibrate for seven to ten days at 5°C. in closed containers. In other cases, moisture levels were adjusted by exposing the lyophilized gluten to a humid atmosphere; or, at 50% moisture and higher, by direct addition of water. Results at different pH levels were usually obtained by mixing gluten with appropriate acetate buffers (2.5 ml. 0.5 *M* buffer for each gram of dry gluten), the entire mixture being heated; pH's

were determined by means of a glass electrode both before and after heating. For all experiments in which salts were added, the glutens were dialyzed at least four days before drying to avoid interference by the salts in subsequent solubility determinations. Glutens were dried by lyophilization and ground to pass through a 40-mesh sieve.

Alteration of the properties of heated gluten was measured by loss of solubility in dilute acetic acid and by the decrease in loaf volume of doughs reconstituted with heated glutens. (Since such changes in the properties of the proteins fall within the definition of denaturation given by Neurath *et al.* (11), this more convenient term will be used in the remainder of this report.) Solubility was determined by the method of Olcott and Blish (1): 5 ml. of 95% ethanol were added to one gram of dried, ground gluten, followed by 25 ml. of 0.1 *N* acetic acid. The sample was then allowed to stand one or two days at room temperature with occasional swirling.[3] After centrifuging, the supernatant liquid was filtered through Whatman No. 4 paper, and aliquots

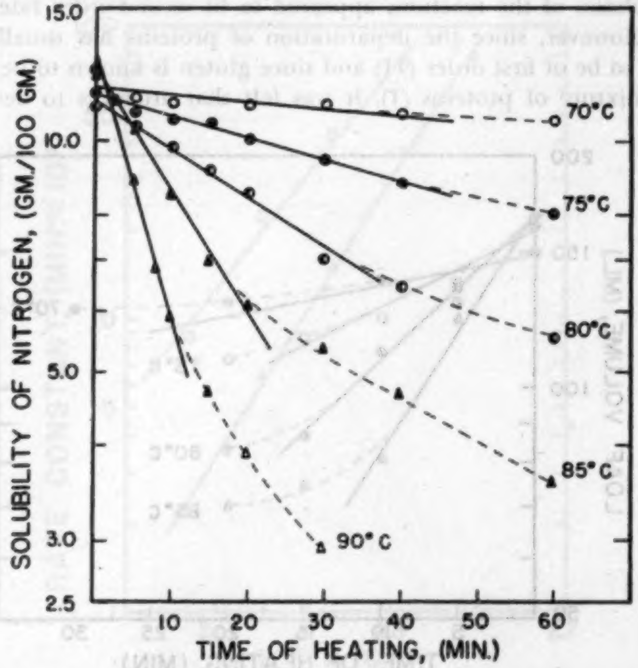

Fig. 1. The denaturation of wet, gum gluten by heat at various temperatures as measured by loss of solubility in dilute acetic acid. Gluten obtained from commercial flour No. 1. Straight lines calculated by method of least squares.

[3] Slightly more uniform and reproducible results were obtained by this procedure than by gentle but continuous, mechanical shaking of the samples for periods up to 18 hours.

were taken for nitrogen determination. Baking tests were performed in duplicate with micro-doughs reconstituted as described previously (13), using the same starch for all baking tests. Amide nitrogen was determined by the method described by Mecham and Olcott (10).

Results

The denaturation of wet, gum gluten by heat was studied at temperatures ranging from 70° to 90°C. using both the solubility and the baking-test methods for measuring changes in the properties of the proteins. The results obtained are shown graphically in Figs. 1 and 2, in which the values are plotted logarithmically against time of heating. Straight lines should be obtained by this method if the course of the reaction has a first-order dependence on concentration of reacting substance, and the slopes of the lines will be equivalent to the first-order rate constants. Values for the initial phases of the reactions, at least, fit the designated lines[4] reasonably closely, but the course of later phases of the reactions appeared to fit second-order rate curves best. However, since the denaturation of proteins has usually been found to be of first order (11) and since gluten is known to be a complex mixture of proteins (1), it was felt that attempts to determine

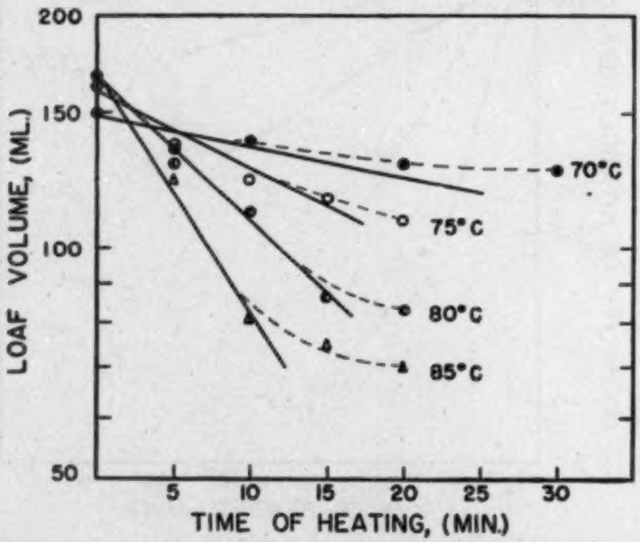

Fig. 2. The denaturation of wet, gum gluten by heat at various temperatures as measured by decrease in loaf volume of reconstituted doughs. Gluten obtained from commercial flour No. 1. Straight lines calculated by method of least squares.

[4] Calculated by the method of least squares.

the order of the reaction more closely were unwarranted in the present experiments. The observed reaction rates are most likely composite values for the various components of the gluten complex.

The range of zero-time values shown in Figs. 1 and 2 are attributed partly to variations in the composition of the glutens, since freshly washed samples were prepared for each series of these particular determinations. It is difficult to prepare samples of gluten, from the same flour, which contain strictly uniform proportions of different components. Additional variation could arise during the drying of the unheated samples by lyophilization. The native gluten would be more sensitive to changes caused by the drying operations than that which had already been altered by the heat treatments.

It is apparent that the baking test is more sensitive than the solubility method for detecting the denaturation of gluten. A major reason for this difference is probably that only about 75% of the total nitrogen of unheated gluten is soluble under the conditions of the solubili-

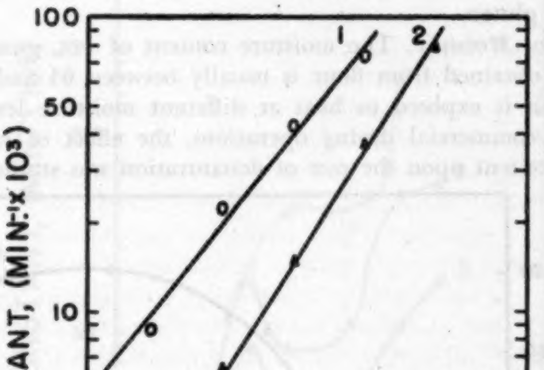

Fig. 3. The relation between temperature and rate of denaturation of wet, gum gluten by heat as measured by the baking-test method (curve 1) and, by the solubility method (curve 2). The energy of activation ($E = \text{slope} \times R$) equals 34,600 calories per mole by the baking test, and 43,800 by solubility.

ty test. Nearly one-fourth of the gluten, therefore, is unavailable for study with the solubility method used, and it is this less-soluble portion of gluten (glutenin) that has been found to be most susceptible to denaturation (2, 14). On the other hand, the smaller rates found with the simpler solubility method permit its use over a wider range of conditions. It is also more accurate for measuring small differences in the extent of denaturation.

Energy of Activation. In Fig. 3 are shown the curves obtained by plotting values of the first-order rate constants from Figs. 1 and 2 on a logarithmic scale against the reciprocal of absolute temperature. The slopes of the lines when multiplied by the gas content ($R = 1.987$ cal./degree/mole) represent the energy of activation as expressed by the Arrhenius equation ($d \ln k/dt = E/RT^2$) (11). A value of 34,600 calories per mole is thus obtained with the baking-test method and 43,800 calories per mole with the solubility method. These values are within the range reported as typical for proteins (11), and illustrate further the greater sensitivity of the baking-test method for detecting changes in gluten.

Effects of Moisture. The moisture content of wet, gum gluten as ordinarily obtained from flour is usually between 64 and 67%, but since gluten is exposed to heat at different moisture levels during baking or commercial drying operations, the effect of variation in moisture content upon the rate of denaturation was studied. The re-

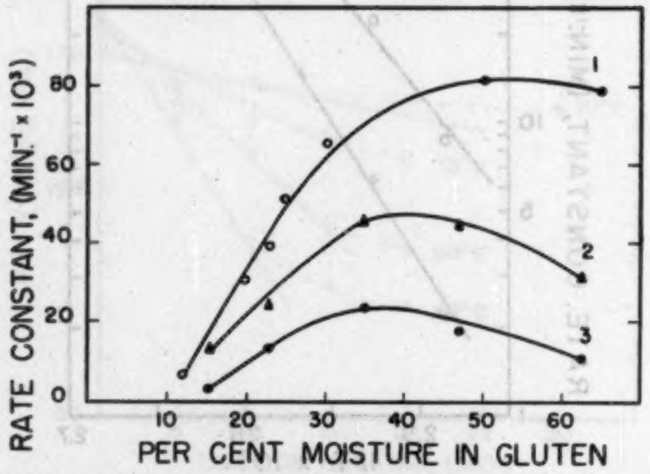

Fig. 4. The effect of moisture content upon rate of denaturation of gum gluten by heat. Curve 1, solubility method on gluten from commercial flour No. 1 heated at 90°C.; curve 2, baking-test method on gluten from commercial flour No. 2 heated at 80°C.; curve 3, solubility method on gluten from commercial flour No. 1 heated at 80°C.

sults obtained are shown in Fig. 4. Measurements with the solubility method (curve 1) show that only small changes in the rate of denaturation occur at 90°C. as long as the moisture content remains above about 35 to 46%. Below this value the rates diminish rapidly until at levels of 4-5% moisture, no measurable denaturation can be detected for heating periods up to one hour. Results obtained at 80°C. with gluten from a second flour show a well-defined maximum by both methods (curves 2 and 3) which is only suggested at the higher temperature. Again, values obtained with the baking-test method (curve 2) are larger at corresponding points than those obtained with the solubility method (curve 3), but the curves are very similar in shape.

Effects of pH. The results in Fig. 5 indicate that the denaturation is a complex function of pH. At pH 4 denaturation proceeds very slowly, but the rate increases rapidly as the pH rises to about 5 (curve

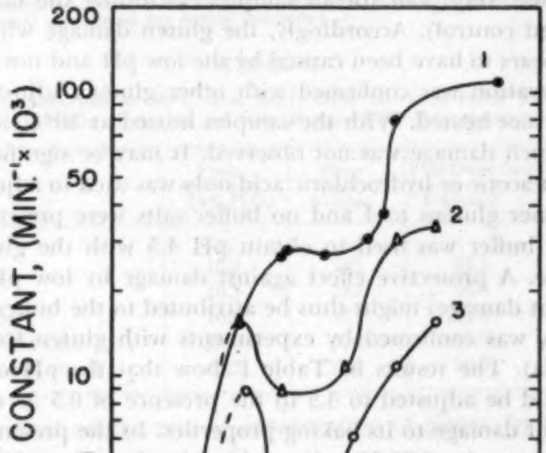

Fig. 5. The effect of pH on rate of denaturation of gluten by heat. Curve 1, solubility method on gluten from commercial flour No. 1 heated at 90°C.; curve 2, baking-test method on gluten from commercial flour No. 2 heated at 80°C.; curve 3, solubility method on gluten from commercial flour No. 2 heated at 80°C.

1). From this point the rate remains virtually constant until at about pH 6 another sharp increase occurs. Between pH 6.5 and 7.5 the rate again becomes fairly constant. The results obtained at 80°C. with gluten from the second commercial flour (curves 2 and 3) are essentially similar except that more pronounced minima in the rate curves occur between pH 4.5 and 6. It is not known to what extent differences in the results with the two glutens are due to inherent differences in the glutens themselves or to the different temperatures, but the similarity in the general shape of the curves is interpreted to indicate that similar relations exist between pH and rate of denaturation at both temperatures.

The very low rates of denaturation observed with the solubility method for gluten heated at 90°C. near pH 4 (curve 1) were confirmed by the baking-test method ($k = 2.7 \times 10^{-3}$ min.$^{-1}$). Although heating caused practically no progressive damage at this pH, baking performance was very poor for all samples (including the unheated but pH-adjusted control). Accordingly, the gluten damage which was observed appears to have been caused by the low pH and not by heating. This observation was confirmed with other glutens adjusted to pH's near 4 but not heated. With the samples heated at 80°C. and pH 4.5, however, such damage was not observed. It may be significant in this regard that acetic or hydrochloric acid only was used to adjust the pH's of the former glutens to 4 and no buffer salts were present, whereas, an acetate buffer was used to obtain pH 4.5 with the gluten in the second case. A protective effect against damage by low pH (but not against heat damage) might thus be attributed to the buffer salts. This effect, also, was confirmed by experiments with gluten from another flour (Mida). The results in Table I show that the pH of unheated gluten could be adjusted to 4.5 in the presence of 0.5 *M* sodium acetate without damage to its baking properties. In the presence of 0.5 *M* sodium acetate plus 0.25 *M* sodium chloride, the pH could be lowered to 4.0 with no decrease in loaf volume.

Effect of Salt Concentration. Salt concentration is sometimes a significant variable in protein denaturation (8). The influence of this factor was therefore determined for the heat denaturation of wet, gum gluten at its normal pH. Two-gram samples of dry gluten were mixed with 4 ml. of sodium chloride solutions ranging in concentration from 0.01 to 3.0 molar and allowed to stand overnight before being heated to 90°C. for five minutes; the pH of all the samples was approximately 6.2. After recovery by dialysis, the glutens showed no significant variation in solubility. Ionic strength, therefore, appeared

TABLE I

EFFECT OF SALTS ON THE DAMAGE TO THE BAKING PROPERTIES OF GLUTEN CAUSED BY LOW pH CONDITIONS

pH of Gluten[1]	Salt Present	Loaf Volume
		cc.
4.0	none	142
4.0	0.5 *M* acetate	136
4.5	0.5 *M* acetate	161
5.0	0.5 *M* acetate	157
6.2	0.5 *M* acetate	158
4.0	0.5 *M* acetate — 0.25 *M* NaCl	161
4.3	0.5 *M* acetate — 0.25 *M* NaCl	165
4.7	0.5 *M* acetate — 0.25 *M* NaCl	163
5.0	0.5 *M* acetate — 0.25 *M* NaCl	163
6.2	none	164

[1] Gluten was adjusted to the pH value shown, allowed to stand 30 minutes, then neutralized and lyophilized. Proof times of the reconstituted doughs were regulated to provide production of equal amounts of gas during this period.

TABLE II

RATES OF DENATURATION AT 80°C. FOR WET, GUM GLUTENS FROM VARIOUS FLOURS AS MEASURED BY SOLUBILITY AND BAKING-TEST DETERMINATIONS

Flour	Flour Protein at 14% H_2O	Flour Loaf Volume	First Order Rate Constant x 10^3	
			Solubility	Baking-Test
	%	*cc.*		
Turkey	10.2	745	12.1	56.6
Pawnee	10.1	785	10.9	42.9
Red Chief	10.3	575	10.9	36.2
Commercial No. 1	10.0	730	14.9	42.4
Commercial No. 2	10.2	660	10.9	31.0

to have no effect on the rate of denaturation of gluten by heat under these conditions.

Results with Other Glutens. The possibility that glutens from flours of different quality might be denatured at different rates was investigated with glutens obtained from Turkey, Pawnee, and Red Chief flours. The glutens were heated at 80°C. and the rate of denaturation as measured by both the solubility and baking-test methods are shown in Table II along with the values already determined for the two commercial flours.

There appears to be no significant variation among the values obtained with the solubility method,[5] but large differences are found

[5] The mean significant difference (at the 5% point) between values obtained from duplicate determinations with one gluten at several temperatures was found to be 4.7×10^{-3} min.$^{-1}$ by this method.

within the greater range of values obtained with the baking-test method. It is noteworthy that the two flours with the lowest loaf volumes (Red Chief and Commercial No. 2) also had the lowest rate constants, but no consistent trend is present.

Denaturation of Gliadin. Because of the complex composition of gluten and because the rate curves shown in Fig. 1 probably represent composite values for the simultaneous denaturation of several proteins, it was of interest to determine separately the rate of denaturation for the gliadin fraction of gluten. Accordingly, 2-g. samples of a gliadin preparation[6] were mixed with 3 ml. of water, sealed in small test-tubes, and heated at 90°C. for various periods of time. The extent of denaturation was measured by the solubility method. The results shown in Fig. 6 indicate the occurrence of an induction period of about ten minutes. After the interval, the denaturation attains a

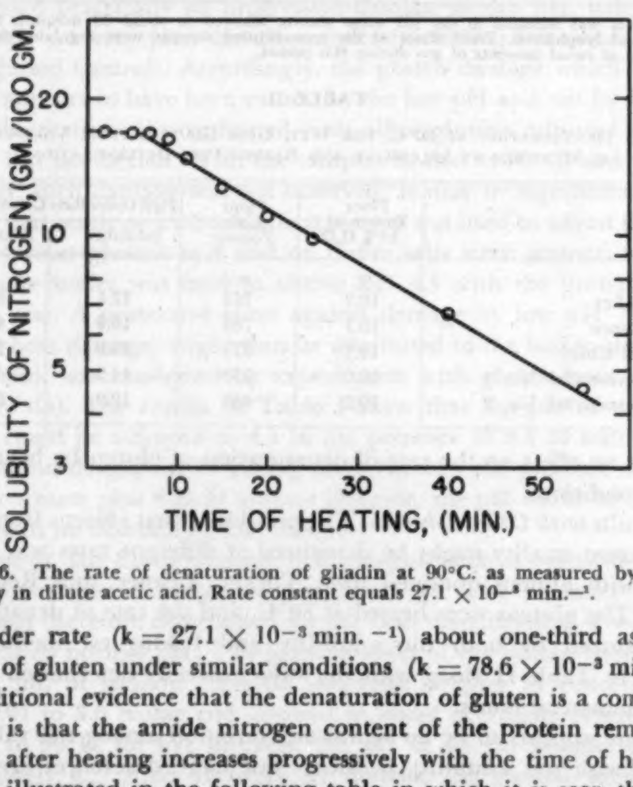

Fig. 6. The rate of denaturation of gliadin at 90°C. as measured by loss of solubility in dilute acetic acid. Rate constant equals 27.1×10^{-3} min.$^{-1}$.

first order rate ($k = 27.1 \times 10^{-3}$ min.$^{-1}$) about one-third as large as that of gluten under similar conditions ($k = 78.6 \times 10^{-3}$ min.$^{-1}$).

Additional evidence that the denaturation of gluten is a composite process is that the amide nitrogen content of the protein remaining soluble after heating increases progressively with the time of heating. This is illustrated in the following table in which it is seen that the

[6] Obtained by extracting flour with 60% ethanol at room temperature and cooling the extract to −30°F.

amide nitrogen content of the soluble material approaches that of gliadin.

Heating time at 90°C.	Soluble N per 100 g. gluten	Amide N as % of Total N in Soluble Fraction
min.	*g.*	
0	13.5	23.4
5	10.2	24.7
10	7.5	25.1
20	4.8	25.7

Discussion

The complex composition of gluten severely limits the theoretical inferences which might be drawn from a detailed study of its denaturation; but the survey of effects of various factors presented here appears to have practical value with respect to problems encountered in the baking of many types of products or in commercial drying operations. The intricate relationships found among temperature, pH, and the rates of denaturation are not at all atypical of proteins. Similar relationships have been recently and intensively studied with other proteins (7, 8, 11), and have been readily explainable in terms of effects on equilibria involved among the reactions leading to denaturation.

The relationship between rate of denaturation and moisture content appears to be somewhat extraordinary, but it must be remembered that even at the higher moisture levels used, a very concentrated system is present and that water may exert effects both as a reactant and as a diluent. It may be noted that Ferry (4) likewise found unusual effects with fibrin films heated over a wide range of moisture contents.

The excellent parallelism of results obtained with the two test methods indicates that the solubility method can be used with confidence for studies limited to the heat denaturation of gluten, even though a considerable part of native gluten is insoluble under the conditions of the test. It is possible that the test can be modified to overcome this particular limitation, but the interference caused by salts in materials being tested might be more difficult to avoid. Dialysis is quite effective for removing salts, but is a troublesome, time-consuming operation with gluten.

The damage to baking properties of gluten found at low pH conditions in the absence of buffer salts and the lack of effect of ionic strength on the rate of denaturation *by heat* suggest the occurrence of a pattern of changes at low pH's quite different from that caused by heating. Although Olcott, Sapirstein, and Blish (12) observed that

the solubility characteristics of gluten proteins were not appreciably altered by brief heat treatments of gluten dispersed in acetic acid at pH's between 3 and 4, Lusena (9) found that the pH of a dispersion of gluten before lyophilization must be maintained above 5 or damage to baking properties of the gluten occurs. This author, however, stressed the necessity of using virtually salt-free solutions in order to obtain a concentration of gluten of practical magnitude. The salt content of gluten, therefore, appears to be of considerable importance for preserving the baking properties of gluten exposed to low pH conditions.

The observed variation among the rates of denaturation, as measured by the baking test, of glutens from flours of different baking quality suggests quantitative differences in the composition of these glutens which are related to the observed quality differences. Denaturation experiments coupled with fractionation may, therefore, become a fruitful method for studying quality variation among flours.

Acknowledgments

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STUDIES ON CRUST COLOR. I. THE IMPORTANCE OF THE BROWNING REACTION IN DETERMINING THE CRUST COLOR OF BREAD.[1, 2]

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ABSTRACT

Flour milled from certain lots of Dutch wheat, yields loaves with a greyish crust color. The insufficient crust color is not due to shortage of sugars and the caramelization of the sugars does not provide an adequate explanation. The normal crust color of bread is chiefly the outcome of reactions of the Maillard type, i. e., reactions between reducing sugars and proteins or their hydrolytic products. Shortage of the latter may cause an insufficient crust color as well as shortage of sugars.

Adding dried egg white to grey-baking flour improved the crust color. The addition of dried egg white and individual amino acids to wheat starch and reducing sugar produced a stated effect on crust color.

A sufficient supply of steam at the start of the baking process is essential for the development of the crust color.

The literature yields few exact data about the reason(s) for the development of the brown crust color of bread. Blish, Sandstedt and Platenius (3) found a positive correlation between the diastatic power of flour and the crust color. Most manuals state that the brown crust color of bread is due to carmelization of sugars (7, 12) or of sugars and dextrins (2), formed during the fermentation process and the early stages of baking. In accordance with this concept, Kent-Jones and Price (8) enumerate a number of factors causing the absence of a brown crust color, which are all related to shortage of sugars. Certain wheat samples under investigation, however, yielded grey-baking flour, though no shortage of sugars could be established.

Many recent investigations deal with the so-called "browning reaction" in foods, which is characterized by the development of a brown color (10) after keeping the foods for a long time at room temperature or after exposing them to high temperatures such as in baking. The presence of proteins or their hydrolytic products on the one hand and reducing sugars on the other is essential for the "browning reaction".

Maillard in 1912 (9) was the first to describe reactions of this type with amino acids. Barnes and Kaufman in 1947 (1) point out the possible importance of browning reactions as a factor in the formation of color in baking and suspect that reactions of this type may also be

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essential for the development of flavors of many foodstuffs, as for instance the bread crust.

The question arose as to whether the normal brown crust color of bread is the outcome of reactions of the Maillard type and if so, whether the grey-baking properties of certain flour samples under investigation could be explained in accordance with this view.

Materials and Methods

All samples of flour, except one, were milled from Dutch wheat on a Bühler laboratory mill. Some of these wheat samples were obtained from the Dutch milling industry and others were known varieties from experimental-fields. A commercial flour of 78% extraction, milled from a grist of Dutch and import wheat was used as a control to provide bread of normal crust color.

Methods of Analysis. Moisture was determined in a 130°C. air-oven for one hour (after the oven regained 130°C.), ash by incineration at 550°C. to a light grey ash, and crude protein (N × 5.7) by the Kjeldahl method using the Parnas-Wagner apparatus.

Maltose figures were determined according to Van der Lee (See Pelshenke, 13), using however Luff's solution (See Browne and Zerban, 5) instead of Fehling's.

Pre-existing maltose was determined by the same procedure, leaving out the incubation period.

Reducing and non-reducing sugars were determined in the bread crust. The crust was separated from the crumb, dried at 80°C., pulverized and extracted for one hour with distilled water. After treating the extract with Carrez solutions (6) and filtration, reducing sugars were determined in an aliquot of the filtrate using Luff's solution. Another aliquot was subjected to inversion with hydrochloric acid at 70°C. for ten minutes, the total reducing sugars determined, and the non-reducing sugars computed by subtracting the quantity of reducing sugars found before inversion.

The pH value of the baking products was determined with a pH-meter (Radiometer, Copenhagen) after thorough mixing of the crumb with a small amount of distilled water.

Baking Methods. In the experimental baking tests, doughs containing 1.75% yeast and 2% salt (flour basis) were prepared with a consistency of 360 Brabender Units. The doughs were fermented at 30°C. and hand punched after 20, 40, and 55 minutes, then placed in the baking pan and proofed for a sufficient time to yield a standard amount of carbon dioxide, as determined by the S.I.A. recorder[4] on

[4] Manufactured by Aktiebolaget S. J. A., Stockholm, Sweden. This fermentation recorder has been described by Pelshenke (14).

an aliquot of the original dough. The doughs were baked for 25 to 30 minutes in an oven at about 250°C.

Two or three loaves were made from each dough. Their sizes varied from 100 - 400 g. in different experiments, depending upon the amount of material available.

Small-scale baking tests were used in several experiments. In one case 30 g. of flour and 0.6 g. yeast was used and the dough kneaded in the mixograph (15). Round tins (2 cm. high, bottom diameter 4 cm., and top diameter 7 cm.) were used.

A number of "model experiments" were carried out by baking mixtures containing wheat starch, water, sugars, dried egg white or amino acids and, in some cases, of leavening agents.

To distinguish easily small differences in grey-baking properties an oven temperature of 210°C. was used in most experiments. At this temperature normal-baking flour yielded loaves with a light golden brown crust, whereas the abnormal flours gave loaves with a greyish-white crust color in extreme cases.

To avoid a drop in the oven temperature which resulted from the opening of the oven door at regular intervals during the early experiments, the fermentation periods were adjusted in such a way that the loaves were all placed in the oven at the same moment. This was done chiefly at the expense of the three first fermentation periods.

Several experiments with different types of flour proved that lengthening and shortening the three first fermentation periods by 12 minutes at the utmost, did not influence the crust color. In many experiments steam was supplied at the start of the baking process, since this made the surface of the loaves glossy and improved the crust color of those made from grey-baking flours.

Unless indicated otherwise in the description of the baking experiments, no steam was supplied at the start of the baking process and an oven temperature of 210°C. was used.

Results and Discussion

Analysis of Flour Samples. Most experiments were carried out with grey-baking flour No. 1, milled from Dutch wheat of unknown variety. This flour was compared with a commercial flour No. 2, which was used as a normal-baking standard flour. Reducing and non-reducing sugars were determined in the crust of loaves baked from these two types of flour. The data recorded in Table I show that there is a great difference in protein content and a maltose figure. A maltose figure

TABLE I

ANALYSIS OF TWO FLOUR SAMPLES AND OF THE CRUST OF THEIR LOAVES

	Sample No. 1, Giving Grey Crust Color	Sample No. 2, Giving Normal Crust Color
Protein,[1] %	8.1	13.2
Maltose figure,[2] %	0.69	1.05
Pre-existing maltose,[2] %	0.27	0.25
Reducing sugars in bread crust,[1] %	1.40	1.59
Non-reducing sugars in bread crust,[1] %	0.71	0.43

[1] On moisture-free basis.
[2] On flour basis.

of 0.69, however, does not point to sugar shortage in cases of flour milled from Dutch wheat on a Bühler laboratory mill. Of 145 flour samples of the 1947 crop, for instance, which included all the important Dutch wheat varieties, 58 had a maltose figure lower than 0.70 but most of these yielded loaves with a normal crust color. The higher maltose figure of flour No. 2 is undoubtedly connected with the fact that this flour was produced on a commercial mill.

The amount of sugars found in the greyish crust of the loaves from flour No. 1, indicates also that there is no shortage of sugars. The caramelization theory provides no satisfactory explanation for the abnormal crust color in this case.

Addition of Gluten to Grey-Baking Flour. To the dough of a grey-baking flour No. 3, milled from Dutch wheat of the variety Alba, gluten was added. This gluten was in one case made from this grey-baking flour No. 3, in a second case from normal-baking flour No. 4, milled from Hard Winter wheat. The gluten was washed out by hand with tap water from the respective doughs. Table II shows the results of the baking experiments. In both cases a considerable improvement in crust color was obtained after the addition of the gluten.

Addition of Wheat Starch to Normal-Baking Flour. Increasing amounts of commercial wheat starch were added to normal-baking flour No. 2. The resulting crust colors were compared with the crust color of loaves from grey-baking flour No. 1. Table III shows that the addition of wheat starch causes a considerable deterioration of the crust color. In all three cases the crust color, after the addition, was inferior to the color produced by the grey-baking flour, although in two cases the protein content was higher. This proves that the pro-

TABLE II

EFFECT ON CRUST COLOR OF THE ADDITION OF GLUTEN TO GREY-BAKING FLOUR

Flour Used	Weight	Addition to the Dough of gluten from	Protein[1] in Dough	Crust Color
	g.		%	
Normal-baking flour No. 4	180	......	11.2	brown
Grey-baking flour No. 3	180	......	7.1	light cream to grey-white
Grey-baking flour No. 3	170	73 g. flour No. 4	10.9	brown with a grey bloom
Grey-baking flour No. 3	170	125 g. flour No. 3	10.7	slightly lighter brown with a grey bloom

[1] On moisture-free basis.

TABLE III

EFFECT ON CRUST COLOR OF THE ADDITION OF WHEAT STARCH TO NORMAL-BAKING FLOUR

Flour Used	Weight	Wheat Starch	Protein[1] in Dough	Crust Color
	g.		%	
Normal-baking flour No. 2	175	..	13.0	light brown
Grey-baking flour No. 1	175	..	7.9	yellow-cream
Normal-baking flour No. 2	121	54 g.	9.2	grey-white with a bit brown
Normal-baking flour No. 2	105	70 g.	8.1	grey-white with a bit cream
Normal-baking flour No. 2	89	86 g.	7.3	grey-white (like moist chalk)

[1] On moisture-free basis.

tein content of the dough is not the only determining factor in crust color.

Addition of Dried Egg White, Sugars and Individual Amino Acids to Grey-Baking Flour. Although gluten is a complicated mixture, it seems obvious to connect its effect in improving crust color with the proteins, these being the major component of gluten. To verify this, experiments were made with dried egg white,[5] which is less complex than gluten.

Table IV gives the results of these experiments, where dried egg

[5] Dried egg white = "Albumen ovi siccum", according to the fifth edition of the "Nederlandsche Pharmacopee".

TABLE IV

EFFECT ON CRUST COLOR OF THE ADDITION OF DRIED EGG WHITE WITH OR WITHOUT SUGARS TO GREY-BAKING FLOUR

Exp.	Flour Used	Weight	Dried Egg White	Sugars Used	Weight	Crust Color
		g.	g.		g.	
A	grey-baking flour No. 1	600	..	....	..	light cream
	grey-baking flour No. 1	600	36	....	..	brown
	grey-baking flour No. 1	600	36	dextrose	12	deep brown
	grey-baking flour No. 1	600	..	dextrose	12	dark cream
	normal-baking flour No. 2	600	..	....	..	light golden brown
B	grey-baking flour No. 1	150	9	....	..	light golden brown
	grey-baking flour No. 1	150	9	dextrose	1.6	golden brown
	grey-baking flour No. 1	150	9	maltose	3	golden brown
	grey-baking flour No. 1	150	9	sucrose	3	golden brown
	grey-baking flour No. 1	150	..	....	..	cream
C[1]	grey-baking flour No. 3	150	9	....	..	dark cream
	grey-baking flour No. 3	150	9	dextrose	1.6	light brown
	grey-baking flour No. 3	150	9	maltose	3	dark cream to light brown
	grey-baking flour No. 3	150	9	sucrose	3	dark cream
	grey-baking flour No. 3	150	..	....	..	grey-white
D	grey-baking flour No. 1	30	..	dextrose	0.32	grey-white to cream
	grey-baking flour No. 1	30	0.3	dextrose	0.32	cream with brown spots
	grey-baking flour No. 1	30	0.6	dextrose	0.32	as many cream spots as brown spots
	grey-baking flour No. 1	30	0.9	dextrose	0.32	light brown with many cream spots
	grey-baking flour No. 1	30	1.2	dextrose	0.32	light brown with few cream spots
	grey-baking flour No. 1	30	1.5	dextrose	0.32	golden brown
	grey-baking flour No. 1	30	1.8	dextrose	0.32	fairly deep golden brown
	grey-baking flour No. 1	30	1.8	....	..	golden yellow
	grey-baking flour No. 1	30	1.8	dextrose	0.08	deep golden yellow
	grey-baking flour No. 1	30	1.8	dextrose	0.16	light golden brown
	grey-baking flour No. 1	30	1.8	dextrose	0.24	golden brown
	grey-baking flour No. 1	30	1.8	dextrose	0.32	fairly deep golden brown
	grey-baking flour No. 1	30	1.8	dextrose	0.64	very deep golden brown
	grey-baking flour No. 1	30	1.8	dextrose	0.96	very deep golden brown
	grey-baking flour No. 1	30	1.8	dextrose	1.28	deep golden brown

[1] No yeast or leavening agent was used in the baking tests.

white and/or sugars were dissolved in water and added to grey-baking flour in making the dough.

Experiment A (Table IV) shows that the addition of dried egg white considerably improves the crust color. This effect is increased distinctly when dextrose is added together with dried egg white, whereas the mere addition of dextrose gives only a slight improvement of crust color.

From the results of experiment B (Table IV) it appears that, added in combination with dried egg white, the color improving effects of equimolecular amounts of dextrose, maltose and sucrose are about the same. The positive effect of sucrose appears to be in contradiction with what is known concerning the browning reaction in other fields. However in doughs, sucrose is rapidly converted to glucose and fructose by the invertase of yeast, as shown by Geddes and Winkler in 1930 (7).

In experiment C (Table IV) no yeast or leavening agent was added to the doughs. Immediately after mixing, the doughs were placed in the baking pans and baked. Under these conditions, where the splitting action of yeast enzymes on the added sugars has been eliminated, dextrose shows a distinct improving effect, maltose a smaller effect and sucrose no positive effect at all. These facts are well in accordance with our knowledge of browning reactions.

In the small-scale baking tests of experiment D (Table IV), increasing amounts of dried egg white in combination with a constant amount of dextrose were added to grey-baking flour No. 1, as well as a constant amount of dried egg white together with increasing amounts of dextrose.

The results demonstrate clearly the importance of both the protein and the reducing sugar for the development of the crust color. Glutamic acid was added to grey-baking flour No. 1 in increasing amounts of 0.5, 1 and 2% on flour basis. The solution of glutamic acid in water was adjusted with sodium hydroxide to a pH of 6.2 and added to the flour on doughmaking. A distinct improvement in crust color, proportional to the amount of the amino acid, was shown.

Addition of 0.5% glycine to grey-baking flour No. 1 also improved the crust color.

"Model Experiments" with Wheat Starch, Different Sugars and Dried Egg White or Amino Acids. Different sugars and dried egg white were dissolved in water and added to wheat starch in doughmaking. Instead of yeast, baking powder was used as a leavening agent. Table V presents the results.

TABLE V

CRUST COLOR DEVELOPED IN BAKING EXPERIMENTS WITH WHEAT STARCH, DRIED EGG WHITE AND DIFFERENT SUGARS

Wheat Starch Used in Doughmaking	Dried Egg White	Sugars Used	Weight	Crust Color
g.	g.		g.	
218	32	. .	. .	grey-white
215	32	dextrose	2.7	brown
213	32	maltose	5	light brown
213	32	sucrose	5	grey-cream
392	. .	maltose	8	greyish

It is apparent from these experiments that a brown crust color is developed when both dried egg white and a reducing sugar (dextrose, maltose) are present, whereas practically no coloring effect appears when only one of these substances has been added to wheat starch or when dried egg white has been added in combination with a non-reducing sugar (sucrose). This proves that under the conditions of these "model experiments" the development of a brown crust color is due to browning reactions. Dextrose has in combination with dried egg white more coloring effect than an equivalent amount of maltose.

In a second series of "model experiments", small-scale baking tests were performed with wheat starch, a constant amount of dextrose and equimolecular amounts of different amino acids. Each dough was made of 25 g. of total solids. With the exception of cystine, all constituent amino acids of egg albumin have been examined (4). When the pH of the amino acid solution was beyond the range 5.1 - 6.3, either diluted hydrochloric acid or sodium hydroxide was added, in order to maintain a narrow pH range of the doughs. No leavening agents were used, these causing ruptures of the crust and local differences in pH. The baking time was fixed at 45 minutes in order to obtain even crust colors. The results are shown in Table VI.

It is apparent from Table VI that the constituent amino acids of egg albumin have different brown-coloring potencies in combination with dextrose.

Influence of Ammonium Compounds on the Crust Color. Browning reactions do not occur only between reducing sugars and amino acids, many other compounds may also be involved. According to Patron (11) for instance, amino acids may be substituted by proteins, polypeptides, amines and ammonium salts.

As indicated before, addition of protein (dried egg white) or amino

TABLE VI

Crust Color Developed in Baking Experiments with Wheat Starch, Dextrose and Equimolecular Amouunts of Different Amino Acids

Amino Acid	Weight	Dextrose	Crust Color[1]
	g.		
Lysine-2 HCl	0.135 (0.159)[2]	0.27 g.	light brown to brown to deep brown
Glycine	0.055	0.27 g.	light brown to brown to deep brown
Serine	0.077	0.27 g.	golden yellow to light brown to brown
Alanine	0.065	0.27 g.	golden yellow to light brown to brown
Arginine	0.127	0.27 g.	golden yellow to light brown to brown
Valine	0.086	0.27 g.	yellow to light brown to brown
Leucine	0.096	0.27 g.	yellow to light brown to brown
Glutamic acid	0.108	0.27 g.	golden yellow to light brown
Tyrosine	0.132	0.27 g.	golden yellow to light brown
Tryptophane	0.149	0.27 g.	yellow to light brown
Phenylalanine	0.121	0.27 g.	yellow to light brown
Methionine	0.109	0.27 g.	yellow to light brown
Threonine	0.087	0.27 g.	yellow to light brown
Histidine-HCl	0.14	0.27 g.	yellow to light brown
Aspartic acid	0.097	0.27 g.	yellow with brown bloom
Proline	0.084	0.27 g.	yellow to golden yellow

[1] Steam supplied at start of baking process.

[2] Due to shortage of material, less amino acid has been added than the required amount which has been recorded in brackets.

acids (glutamic acid, glycine) to grey-baking flour No. 1, distinctly improved the crust color. Table VII shows that the addition of different ammonium compounds to grey-baking flour No. 1, also improves the crust color. The pulverized salts were thoroughly mixed with the flour before doughmaking. The added amounts of ammonium chloride–sulfate and–phosphate were equivalent to 0.5% ammonium bicarbonate (based on the nitrogen content).

TABLE VII

Effect on Crust Color of the Addition of Ammonium Salts to Grey-Baking Flour No. 1

Grey-baking Flour No. 1	Ammonium Salt Added on Basis of Flour	Amount	Crust Color[1]	pH of Crumb
g.		%		
85			yellow to golden yellow	5.55
85	ammonium bicarbonate	0.5	golden yellow to light brown	7.49
85	ammonium bicarbonate	1.0	light brown to brown	7.88
85	ammonium chloride	0.34	light brown to brown	5.38
85	ammonium sulfate	0.42	light brown to brown	5.35
85	di-ammonium phosphate	0.42	light brown	6.28

[1] Steam supplied at start of baking process.

In a series of "model experiments", the same ammonium salts in combination with a constant amount of dextrose were added to wheat starch. Three amino acids were also added for comparison. The amounts of ammonium salts and amino acids were based on the nitrogen content equivalent to an addition of 0.5% ammonium bicarbonate. After dissolving the ammonium salts or amino acids in water, dextrose was added and the solution mixed with wheat starch on doughmaking. No leavening agents were used. The results are presented in Table VIII.

TABLE VIII

CRUST COLOR IN BAKING EXPERIMENTS WITH WHEAT STARCH, DEXTROSE AND AMMONIUM SALTS OR AMINO ACIDS

Ammonium Salt or Amino Acid[1]	Amount	Dextrose[1]	Crust Color[2]
	%	%	
..		1.07	light cream
ammonium bicarbonate	0.5	1.07	cream
ammonium chloride	0.34	1.07	cream with light brown spots
ammonium sulfate	0.42	1.07	cream with brown spots
di-ammonium phosphate	0.42	1.07	brown to deep brown
glycine	0.47	1.07	deep brown
serine	0.66	1.07	deep brown
phenylalanine	1.04	1.07	brown with golden yellow bloom

[1] On basis of total solids.
[2] Steam supplied at start of baking process.

Further "model experiments" with ammonium salts, with and without adjusting the pH of the solution of the ammonium salt and dextrose to 5.5, showed that the apparent pH differences did not influence the crust color.

The results of these experiments with ammonium compounds are another indication that browning reactions are involved.

Influence of Steam on the Crust Color. Baking experiments with grey-baking flour No. 1, to which dried egg white and/or dextrose had been added, were performed without steam and with steam supplied immediately at the start of the baking process. The results are shown in Table IX.

Apart from the fact that the crust is dull without steam and glossy with steam, Table IX shows that steam supplied at the start of the baking process considerably improves the crust color of the grey-baking flour with and without dextrose, whereas practically no im-

TABLE IX

EFFECT ON CRUST COLOR OF THE ADDITION OF STEAM IN BAKING EXPERIMENTS WITH GREY-BAKING FLOUR

Grey-baking Flour No. 1	Dried Egg White[1]	Dextrose[1]	Treatment	Crust Color
g.	%	%		
85	..	..	no steam	grey-white with a bit cream, dull
85	..	1.07	no steam	cream with violet bloom, dull
85	6	..	no steam	golden brown, dull
85	6	1.07	no steam	deep brown, dull
85	..	..	steam	cream with golden yellow bloom, glossy
85	..	1.07	steam	light golden brown, glossy
85	6	..	steam	golden brown, glossy
85	6	1.07	steam	deep brown, glossy

[1] On basis of flour.

provement appears if dried egg white, whether or not in combination with dextrose, has been added.

To investigate whether the color improving effect of an immediate steam supply is due to the promotion of a caramelization process or of a browning reaction, baking experiments were carried out using wheat starch to which increasing amounts of dextrose had been added. No yeast or leavening agents were used. Browning reactions were practically eliminated under these conditions. The results appear in Table X.

TABLE X

EFFECT ON CRUST COLOR OF THE ADDITION OF STEAM IN BAKING EXPERIMENTS WITH WHEAT STARCH AND DEXTROSE

Wheat Starch	Dextrose[1]	Treatment	Crust Color
g.	%		
85	..	steam	grey-white
85	1	steam	grey-white
85	2	steam	grey-white
85	4	steam	grey-white

[1] On basis of wheat starch.

These results make it improbable that the color improving effect of steam should be due to the promotion of a caramelization process. If steam supply at the start of the baking process, however, promotes browning reactions, the question arises why this improvement in crust

color fails to appear after the addition of dried egg white to grey-baking flour No. 1. A possible explanation is that browning reactions in the loaf surface are inhibited if the moisture content decreases too quickly. An immediate supply of steam will retard the evaporation from the loaf surface, which could explain its positive effect on the crust color in cases of grey-baking flour with or without the addition of dextrose. The addition of dried egg white must, due to its water binding capacities, also retard the desiccation of the loaf surface, thus possibly promoting browning reactions indirectly, apart from its direct role as a reacting compound. On this basis it is understandable that supplying steam at the start of the baking process causes no further improvement in crust color, if dried egg white has been added to grey-baking flour.

The baking experiments referred to in Table X were repeated in one case by fixing the baking time at 50 minutes and in another by using an oven temperature of about 240°C. A slight improvement in crust color, proportional to the amount of dextrose added, could be observed in both cases. The small improvement in crust colors, however, make it clear that caramelization processes play no important part under these conditions.

In further experiments with grey-baking flour, a gradual addition of steam has been compared with an immediate steam supply at the start of the baking process. This gradual addition was obtained by injecting small amounts of steam in the oven, 1, 6, 11, 16 and 21 minutes after the start of the baking process.

The results showed that a gradual addition of steam during the baking process has a much smaller improving effect on the crust color of grey-baking flour than immediate steam supply at the start of the baking process.

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EFFECT OF COMMERCIAL FERTILIZERS AND GREEN MANURE ON YIELD AND NUTRITIVE VALUE OF WHEAT. II. NUTRITIVE VALUE WITH RESPECT TO GENERAL COMPOSITION, THIAMINE, NICOTINIC ACID AND THE BIOLOGICAL VALUE OF THE PROTEIN OF GRAIN[1]

G. S. BAINS[2]

ABSTRACT

Application of superphosphate in a dosage of 25 lbs. of phosphoric acid (P_2O_5), per acre, either alone or with green manure *(Cajanus indicus)* produced grain of low average protein content, viz., 9.3%, but higher ash content. Supplementation of the superphosphatic treatments with ammonium sulphate at the rate of 60 lbs. nitrogen per acre increased considerably the protein content of the grain which averaged 10.5%. Animal experiments showed that there was no noticeable effect of the variations in the protein content of samples as a result of fertilization on the biological value of the protein determined by the "balance sheet method". However, the amount of available or "net protein" was comparatively more in the grain characterized by higher protein content as a result of fertilization. The results of thiamine and nicotinic acid assays did not reveal any striking differential effect of fertilization on these constituents.

In the previous paper (2) results of studies regarding the effect of potassium nitrate, superphosphate and superphosphate plus ammonium sulphate, applied in doses of 25 lb. phosphoric acid (P_2O_5) and 60 lbs. nitrogen (N_2), per acre, respectively with and without a

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5 ton supplement of green manure *(Cajanus indicus)* on yield and nutritive value with respect to total phosphorus, phytic phosphorus, non-phytic phosphorus and calcium content of the grain were presented. The present paper deals with the results of further studies regarding the effect of these fertilizer treatments on the ash, fiber, ether-extractives, protein, thiamine, nicotinic acid, biological value, and "digestibility coefficient" of the protein in samples of the fertilized grain.

Materials and Methods

The material employed in this study was basically the same as was used in Part one of this investigation (2). It comprised wheat C 409 sown in four randomized blocks of eight plots, each of which were given different fertilizer treatments. Representative samples of the plots pertaining to various fertilizer treatments were subjected to chemical analysis individually for constituents such as moisture, ash, fiber, fat, protein and nicotinic acid, whereas only composite samples were used for studying the biological value of the protein and for estimation of the thiamin content of the grain.

Moisture, ash, and fiber were determined by the standard A.O.A.C. methods (13). Fat was estimated by extracting the powdered material with petroleum ether (bp. 40–60°C.). Protein content ($N \times 5.7$) was determined by the Gunning-Arnold modification of the Kjeldahl method. Nitrogen-free extract figures were arrived at by difference.

Thiamine was estimated chemically by the well-known thiochrome method of Jansen (11) as used by Aykroyd *et al.* (1).

Nicotinic acid was determined chemically in aqueous extracts of the ground material by the method developed by Swaminathan (17).

For the biological evaluation of the nutritional quality of the protein in the various samples, the formulas and technique based upon the "balance sheet" method of Mitchell (15) as modified and used by Chick *et al.* (7, 8), Boas-Fixen (3), Boas-Fixen and Jackson (4) and Chick and Roscoe (9) were followed. The experimental diets shown in Table I were fed to a group of six adult male albino rats, for a period of seven days in each case. These diets contained 5% protein furnished by wheat (9.3% moisture) and vitamin B complex was supplied by giving each rat 3 ml. of yeast extract (equivalent to 0.75 g. dry yeast) daily.

To start with, all the rats received the nitrogen-free diet (No. 9) for a period of seven days. Feeding with this diet was repeated after finishing with three test diets, viz., No. 1, 3 and 5. The first three

TABLE I
COMPOSITION OF THE EXPERIMENTAL DIETS

Diet No.	Fertilizer treatment[1]	Wheat	Starch	Sugar	Cocoa-nut oil	Salt mixture (Mendels)	Cod liver oil	Calcium carbo-nate
		%	%	%	%	%	%	%
1.	Control (no manure)	48.5	22.7	8.0	13.0	5.0	2.0	0.8
3.	Potassium nitrate	45.3	25.9	8.0	13.0	5.0	2.0	0.8
5.	Superphosphate	55.5	15.7	8.0	13.0	5.0	2.0	0.8
6.	Superphosphate, green manure	54.5	16.7	8.0	13.0	5.0	2.0	0.8
7.	Superphosphate, ammonium sulphate	48.2	23.0	8.0	13.0	5.0	2.0	0.8
8.	Superphosphate, ammonium sulphate, green manure	46.2	25.0	8.0	13.0	5.0	2.0	0.8
9.	Nitrogen-free diet	00.0	71.2	8.0	13.0	5.0	2.0	0.8

[1] Rate of application—Nitrogen (N_2), 60 lbs.; phosphorus (P_2O_5), 25 lbs.; green manure, 5 tons; per acre.

days of each experimental period including the nitrogen-free diet were regarded as preparatory in order to accustom the animals to the diets. Urine and faeces were collected daily during the remaining period of four days. The animals were allowed rest and fed on stock diet for four days after each experimental period, i.e., including feeding with the nitrogen-free diet as well as the test diets. Weighed amounts of diet made into a paste were given to each rat, care being taken that the amounts given were always in excess of the daily average requirement of the animals. The unconsumed food residues were collected daily and dried in the oven at 100°C. From the total intake of food, the intake of nitrogen was calculated. The urine was collected in glass bottles containing 25 ml. of 5% sulfuric acid solution and 2% phenol solution as preservatives. The urine was filtered through cotton wool, made to volume, and an aliquot was taken for nitrogen estimation. The faeces were moistened with dilute oxalic acid solution, dried in the oven, weighed, ground and the nitrogen content determined. The values for 'available or net protein' content of the grain were computed as follows:

$$\text{Crude protein in the grain } \% \times \frac{\textit{Biological value}}{100} \times \frac{\textit{Digestibility Coefficient}}{100}$$

(6, 14, 18, 19, 20).

Results and Discussion

Effect of Fertilization on General Composition. The moisture, ash, fiber, protein, and nitrogen-free extract of the grain obtained from

plots given different fertilizer treatments are shown in Table II. In the last column figures are given for output of protein per acre; these were computed from the yield data reproduced from the first paper in this series (2).

TABLE II
EFFECT OF FERTILIZATION ON THE GENERAL COMPOSITION OF WHEAT[1]

No.	Fertilizer treatment	Mean composition						
		Fat	Fiber	Ash[2]	Protein[2] (Nx 5.7)	Nitrogen free extract	Yield per acre	Output of protein per acre
1.	Control	1.7	2.5	1.57	10.3	74.6	664.0	68.4
2.	Green manure	1.7	2.5	1.57	11.0	73.9	736.0	81.0
3.	Potassium nitrate	1.7	2.5	1.59	11.3	73.6	873.6	98.7
4.	Potassium nitrate, green manure	1.8	2.5	1.55	11.5	73.4	789.6	90.8
5.	Superphosphate	1.7	2.5	1.61	9.2	75.7	992.0	91.3
6.	Superphosphate, green manure	1.7	2.5	1.77	9.4	75.3	1084.0	101.9
7.	Superphosphate, ammonium sulphate	1.7	2.5	1.71	10.5	74.3	928.0	97.4
8.	Superphosphate, ammonium sulphate, green manure	1.7	2.6	1.67	11.1	73.6	1012.0	112.3

[1] The moisture content averaged 9.3%.

[2] Observed values of the variance ration "F", viz., $\frac{\text{variance due to fertilizer treatments}}{\text{variance due to error}}$ for ash and protein are: 6.3 and 14.8 respectively. For statistical significance at 1% level required value of "F" is only 3.64.

There is not much variation in the moisture, fat and crude fiber content of the grain grown under different manurial conditions. The ash content of the grain from plots receiving non-phosphatic fertilizers, viz., treatment Nos. 1, 2, 3 and 4, was almost the same while that of the grain fertilized with phosphate was generally higher. Murphy (16) and Bayfield (5) have also observed that phosphatic fertilization usually increased the ash content of the grain. Application of superphosphate in conjunction with green manure produced grain of relatively higher ash content, i.e., 1.77%, as against 1.55 and 1.57% respectively, for the potassium nitrate and control plots. The differences in the ash content of the various samples as a result of fertilization are statistically significant.

The various fertilizer treatments produced very marked and statistically significant differences in the protein content of grain. Application of superphosphate either alone or with green manure pro-

duced grain of rather low protein content, viz., 9.2% to 9.4%, but in view of the spectacular effect on yield as a result of applying superphosphate with green manure (Treatment No. 6), the calculated output of protein in the grain per acre was 101.9 lbs. per acre as compared with 68.4 for the control. Supplementation of superphosphate with ammonium sulphate increased the protein content of the grain. Incorporation of green manure with the latter treatment (No. 7) exercised a beneficial effect upon both protein content and yield, resulting in a correspondingly higher outturn of protein. The low protein outturn of the control plots is indicative that nitrogen is a limiting factor. The increase in grain yield obtained by fertilization with superphosphate (Treatments 5 and 6) is accompanied by a decrease of approximately 1% in the protein content of the grain. However, the application of ammonium sulphate in conjunction with superphosphate (Treatment No. 7) fully restores the protein content of the grain to that of the control. The overall effect of green manure applications on the protein content of the grain was not spectacular but the outturn of protein in the grain per acre was enhanced in the case of all but one of the treatments.

Effect of Fertilization on the Nutritive Value of Protein. In view of the statistically significant differences encountered in the protein content of grain from plots given different fertilizers, it was considered advisable to ascertain if there were any adverse or beneficial effect of fertilization on the Biological Value (B.V.), Digestibility Coefficient (D.C.), etc., of the protein of grain. The results are summarized in Table III.

TABLE III

EFFECT OF FERTILIZATION ON THE NUTRITIONAL QUALITY OF PROTEIN OF WHEAT GRAIN

No.	Fertilizer treatment	Biological Value (B. V.)	Digestibility Coefficient (D. C.)	Available or "Net protein" content.
		%	%	%
1.	Control	70	90	6.5
3.	Potassium nitrate	67	90	6.8
5.	Superphosphate	68	95	5.9
6.	Superphosphate, green manure	66	98	6.1
7.	Superphosphate, ammonium sulphate	71	90	6.7
8.	Superphosphate, ammonium sulphate, green manure	72	89	7.1

The range of variation in the biological values is not very spectacular and there is not much to choose in favor of a particular fertilizer treatment when the test samples were fed at the same protein level, viz., 5%. However, the digestibility coefficient of the protein of the low-protein grain yielded by treatments Nos. 5 and 6 is slightly higher. The available or "Net Protein Values", show that the protein content of the wheats (Table II) influences the magnitude of these values. In general, therefore, fertilization with a nitrogenous manure, e.g., potassium nitrate or ammonium sulphate in conjunction with superphosphate augments the "available or net protein" contents of the grain chiefly because of its influence on the total protein content.

Effect of Fertilization on Thiamine and Nicotinic Acid. Results of thiamine and nicotinic acid assays of the various samples are presented in Table IV.

TABLE IV
EFFECT OF FERTILIZATION ON THIAMINE AND NICOTINIC ACID CONTENT OF THE GRAIN

No.	Fertilizer treatment	Thiamine [1]	Nicotinic [2] acid
		µg./g.	mg.%
1.	Control (No manure)	3.4	4.2
2.	Green manure	. .	4.2
3.	Potassium nitrate	3.9	3.7
4.	Potassium nitrate, green manure	3.6	3.5
5.	Superphosphate	3.2	3.8
6.	Superphosphate, green manure	3.8	4.3
7.	Superphosphate, ammonium sulphate	4.3	4.0
8.	Superphosphate, ammonium sulphate, green manure	3.9	4.0

[1] Analysis relates to composite samples and so results are not amenable to statistical resolution.
[2] Observed value of "F" is 2.15 as compared with 2.49 required for statistical significance at 5% level.

Statistical examination of the nicotinic acid values for individual plots by the analysis of variance revealed that the differences in the average values pertaining to different fertilizer treatments are not significant. Likewise there is relatively little variation in the average thiamine values of the grain. These observations are in line with the findings of Harris (10), Scheunert and Schieblich (21), Scheunert and Wagner (22) and Leong (12) and support the view that soil and fertilizer conditions do not exercise any marked effect on the thiamine content of cereals.

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COMPARITIVE EVALUATION OF SOME METHODS FOR ESTIMATION OF THE QUALITY OF ACTIVE DRY YEAST[1]

H. J. PEPPLER AND F. J. RUDERT

ABSTRACT

Application of the methylene blue staining procedure and agar plate counts to Active Dry Yeast (ADY) has disclosed that neither reliably differentiates between samples of ADY with relatively high but significantly different baking strength.

The temperature and time of reconstitution of ADY markedly influenced the live cell count as well as the performance of the yeast in normal straight dough fermentations. Rehydration of ADY at low temperatures, 40° to 60°F. (4.4° to 15.6°C.), resulted in low live cell counts, as revealed by both viability test methods, and exhibited poor performance in the bake test. ADY suspended in water at 130°F. (54°C.) showed a high proportion of unstained cells (89%) but produced the poorest bake (60% of the activity of the comparison standard).

The initial temperature of rehydration of ADY exerted the greatest influence on yeast stainability and baking activity. However, ADY reconstituted initially at low temperatures partially recovered its desirable properties when aliquots were warmed moderately. In comparison, reconstitution of ADY at high temperatures, 100° to 110°F. (37.8° to 43°C.), followed by moderate cooling permitted better retention of activity.

Substitutive methods purported to yield presumptive evidence correlating with the baking test have gradually gained acceptance as determinants of yeast activity. Cultural, tinctorial and gasometric techniques are some of the "simplified" procedures in use to ascertain the probable baking strength of bakers' yeast. With the increasing replacement of compressed yeast with Active Dry Yeast, a product of different permeability characteristics, such vitality tests require reevaluation. In this paper, studies are reported of the utility of the methylene blue staining procedure and the agar plate count as measures of the value of active dry yeast for baking purposes.

Materials and Methods

Active Dry Yeast was used in all experiments. Except for samples of ADY of different bake activity selected to check the reliability of the staining reaction, all studies employed ADY of the same lot.

Of the many dyes exhibiting the property of staining "non-viable" cells while "viable" cells remain uncolored (3, 5, 6, 8), methylene blue has been applied most frequently to yeast. For this study cri-

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teria previously established (2) and applied to yeast (7, 9) served as a basis for the methylene blue procedure which was adopted. Preliminary experiments revealed that the least variance occurred when 0.5 g. ADY was suspended in 9.5 ml. tapwater at a controlled temperature held for 10 minutes with mild, continuous agitation. Of this suspension 0.5 ml. was mixed with 39.5 ml. methylene blue (1–10,000) in 0.2 *M* phosphate buffer (pH 4.6) held at 75°F. (23.9°C.) with mild agitation for 30 minutes. An aliquot of the yeast-dye mixture was introduced into a Petroff-Hausser counting chamber by capillarity. The dilution employed with the sample of ADY selected permitted a rapid count of twenty fields resulting in a total count of approximately 1,000 cells. Based on the assumption that the inclusion of a control sample for each series of determinations minimizes the day to day variation, the standard deviation of a single determination is 1.8%. At the level of 95% probability, a single determination is ±3.7% of the true value; duplicate determinations average ±2.6% of the true value; and the average difference between duplicates in a large number of experiments is 2.1%.

For the plate counts 1.0 g. ADY was suspended in 99 ml. 0.1 *M* phosphate buffer at pH 7 at 110°F. (43°C.) for 15 minutes and then agitated 5 seconds in a Waring blendor. All subsequent dilutions were made at the initial reconstitution temperature. Plates were poured with nutrient agar fortified with 1% glucose.

Baking activity was ascertained by determining the total time required for three rises to a volume of 1000 ml. each and a pan proof to a fixed height in the usual straight dough fermentation prepared with ADY added at the rate of 2.7 g./400 g. flour. The total time obtained for the experimental yeast was expressed as a percentage of that observed for a standard yeast under fixed conditions of fermentation.

Results

The pH of the methylene blue solution and the time of contact of yeast and dye influenced the viability count. When rehydration of ADY at 100°F. (37.8°C.) for 15 minutes was followed by exposure to dye solutions buffered at pH 4.6 and 7.1 for 10, 25, 40 and 55 minutes, the comparison revealed that prolonged exposure to methylene blue at pH 7.1 resulted in increasing dead cell counts while the length of contact with the dye at pH 4.6 had no appreciable effect on the number of cells stained:

pH of Dye-Buffer Solution	*Contact Time*[1] *Minutes*	*Unstained Cells* *%*
4.6	10	89.9
	25	92.0
	40	91.2
	55	88.8
7.1	10	90.9
	25	90.0
	40	84.2
	55	79.6

[1] ADY suspended in tapwater at 100° F. (37.8° C.) and held for 15 minutes.

These results are in accord with earlier observations reported for bakers' yeast (1, 2, 7).

The temperature at which ADY was rehydrated in tapwater and maintained for short periods before exposure to the dye greatly affected the stainability of ADY. As shown in Table I, temperatures as low as 40° to 60°F. (4.4° to 15.6°C.) or as high as 135°F. (57°C.) during rehydration resulted in the greatest number of stained cells.

TABLE I

STAINABILITY OF ADY

REHYDRATED AT DIFFERENT TEMPERATURES

BEFORE CONTACT WITH METHYLENE BLUE

Rehydration Temperature	Unstained Cells—Rehydration time[1]		
	5	10	15
°F.	%	%	%
40	33.7	31.2	33.1
60	76.6	72.7	76.0
80	81.7	95.1	93.6
90	87.4	91.0	96.0
100	93.2	88.9	88.9
110	90.3	89.1	86.5
120	88.5	90.0	88.4
130	84.4	88.1	86.4
135	80.0	82.0	71.3

[1] Minutes held at specified temperature before contact with dye solution.

When the initial temperature of reconstitution was followed by various periods at higher or lower temperatures, differences in stainability were predicated chiefly by the initial conditions imposed (Table II). At the lower temperatures of reconstitution, the greatest percent-

age of stained cells was observed. When moderate secondary holding temperatures followed the initial exposure, some recovery could be noted. ADY initially reconstituted at moderate temperatures could be held for long periods at low temperatures without exhibiting marked variations in the methylene blue count.

TABLE II

EFFECT OF TEMPERATURE CHANGES DURING RECONSTITUTION OF ACTIVE DRY YEAST ON STAINABILITY BY METHYLENE BLUE

Rehydration Conditions				
Initial		Secondary		Unstained Cells
° F.	Min.	° F.	Min.	%
40	5	110	10	78.6
40	5	—	—	50.0
55	5	110	10	82.9
100	5	—	—	88.7
100	5	60	5	88.0
110	5	—	—	90.9
110	5	50	15	88.5
110	5	70	15	89.1
110	15	70	30	91.6
110	15	70	120	90.2
110	5	90	10	90.0

Samples of active dry yeast selected from lots known to have different baking activities were reconstituted and held at 110°F. (43°C.) for 15 minutes, the usual conditions employed to prepare ADY for baking. The following representative results disclose the inability of the methylene blue test to differentiate between yeast samples of high but significantly different baking strength:

Sample	*Unstained Cells*	*Relative Bake Activity*[1]
	%	%
1	91.3	100
2	91.4	103
3	92.2	96
4	82.3	85
5	76.4	80
6	80.4	66

[1] Based on the total time of three rises to 1000 ml. volume each rise and a pan proof in the usual straight dough fermentation. The total time obtained for experimental samples was expressed as a percentage of that observed for a standard yeast tested in a similar controlled manner.

Widespread use of poured agar plates to assay the live count of active dry yeast suggested supplementation of some of the earlier

observations with the methylene blue test. Therefore, the extent to which conditions of dilution and plating modified the live count of active dry yeast was determined. Dispersal of the sample was accomplished more effectively when phosphate buffer at pH 7 replaced tapwater as the dilution medium. Acidification of nutrient-glucose agar to pH 4.5 with citric acid prior to pouring the plates resulted in lower live cell counts in comparison with those observed when plates were poured with neutral agar:

Dilution Medium[1]	*pH of Agar*[2]	*% Relative Plate Count*
Tapwater pH 5.5	6.8	100
Tapwater pH 5.5	4.5	87
0.1 M Phosphate Buffer pH 7	6.8	114
0.1 M Phosphate Buffer pH 7	4.5	89

[1] All samples held at 110° F. (43° C.) for 15 minutes.
[2] Nutrient agar containing 1% glucose.

Greatest reductions in plate count were observed when the temperature of the medium of rehydration and dilution was maintained appreciably higher or lower than 110°F. (43°C.) for similar periods of preparation. The results are:

Temperature of Buffer[1]	*Relative Plate Count*
° F.	%
110	100
130	72
60	66

[1] 0.1 M phosphate at pH 7.

A comparison of plate counts of ADY of widely different baking activity disclosed a relationship similar to that noted in the studies of the methylene blue viability test. The following comparison shows that the plate count fails as a reliable index of baking strength, particularly in aged samples:

Sample	*Bake Activity*	*Relative*[1] *Plate Count*
	%	%
1	100	100
2	102	110
3	96	77
4	89	90
5	88	81
6	85	76
7	84	55
8	75	88
9	66	54

[1] Based on the average of several determinations.

The baking test itself is influenced measurably by the temperature and time of reconstitution of ADY. Yeast soaked at 80° or 110°F. (26.7° or 43°C.) decreased in baking strength with extended holding periods. Temperature changes occurring subsequent to an initial reconstitution temperature of 110°F. (43°C.) were not deleterious to the baking activity of active dry yeast; however, as Table III reveals, rehydration and holding at 60°F. (15.6°C.) followed by an increase to 110°F. (43°C.) for a reasonable period failed to produce a satisfactory bake test. Noteworthy is the concordance of data of the baking test and the methylene blue test when the active dry yeast was

TABLE III

EFFECT OF TEMPERATURE OF REHYDRATION ON BAKING STRENGTH OF ACTIVE DRY YEAST

Rehydration Conditions		Relative Activity [1]
° F.	Min.	%
110	15	100
110	30	99
110	45	94
110	5 (then cooled to 70° F., 15 minutes)	103
110	5 (then cooled to 90° F., 15 minutes)	103
80	30	100
80	45	99
80	60	97
60	15	73
60	5 (then raised to 110° F., 15 minutes)	88
130	15	60

[1] Rehydration at 110° F. (43° C.) for 15 minutes selected as standard for comparison.

rehydrated at 60°F. (15.6°C.)—both indicative of poor performance. The methylene blue procedure failed to disclose, however, that active dry yeast rehydrated at 130°F. (54°C.) (Table I) would result in the poorest bake. This was confirmed in a number of replicate experiments.

Discussion

The utility of staining and plating tests is dependent upon the standardization of controlled procedures and the extent to which the results differentiate between yeast samples of different baking activity. Comparative studies reveal that the cultural and tinctorial methods can be standardized readily yielding reproducible data; however, the

limits of interpretation are very narrow. At best both methods allow a classification of yeast samples as good or poor, but they offer no generally reliable estimate of qualities which the bake test measures.

The baking activity of yeast is the resultant of at least two properties: the initial enzymatic activity and the ability of the cells to increase in total effective enzyme concentration. It is conceivable that a viable yeast might not bake well and that an excellent baking yeast might have low reproductive powers. It is known that the yeast population increases significantly in the usual dough (4).

Such general considerations serve to emphasize the difficulties which are involved in reaching a definite conclusion concerning the general utility of staining techniques. From the results reported above with active dry yeast it is clear that data obtained with staining and cultural procedures are only indicative of one of the properties that contribute to activity in the dough. Stainability correlates very roughly with baking activity under some conditions, but under others no relationship can be established.

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AN ALKALINE VISCOSITY TEST FOR SOFT WHEAT FLOURS[1]

KARL F. FINNEY AND WILLIAM T. YAMAZAKI[2]

ABSTRACT

An alkaline viscosity test using sodium bicarbonate instead of lactic acid is described. Alkaline and acid viscosity values are correlated with cookie diameters. Correlation coefficients for alkaline viscosity vs. cookie diameter of two sample groups were —0.93 and —0.98; whereas the r values for lactic acid viscosity vs. cookie diameter were —0.81 and —0.83, respectively. Standard errors of estimate for alkaline viscosity vs. cookie diameters were only 37 to 64% of those involving acid viscosity, and the differentiation or spread between varieties by alkaline viscosity was about twice that by the acid test.

A test commonly used for evaluating the suitability of experimental and commercial soft wheat flours for the production of cookies, cakes, crackers, and pastry goods is the acid viscosity test (2) in its several modifications such as the no-time, one-hour digestion, and 2-g. protein tests (3). It has proven to be of value but has not been consistently reliable as an index of soft wheat quality. The doughs or batters prepared in the production of most baked products from soft wheat flours have a pH value greater than seven due to the chemical leavening agents which are employed (5). Hence, reactions involving the various flour constituents are taking place in an alkaline medium from the time of mixing to the completion of the baking process. Thus, it would seem logical to expect that an alkaline viscosity test would more accurately reflect the reactions that occur between the flour components responsible for quality and certain of the ingredients added in the baking formula, and thereby would be a better index of soft wheat quality than the acid viscosity test now being used. This paper describes a viscosity test that is carried out in a slightly alkaline medium by using sodium bicarbonate instead of lactic acid. The results obtained by both this method and the no-time lactic acid procedure are correlated with diameters of cookies baked by a micro sugar-snap cookie test previously reported (4).

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[2] Hard Winter Wheat Quality Laboratory, Kansas Agricultural Experiment Station, Manhattan, Kansas and Soft Wheat Quality Laboratory, Ohio Agricultural Experiment Station, Wooster, Ohio, respectively.

Materials and Methods

The relationship between alkaline viscosity, adjusted to 9% protein, and cookie diameter was tested on 44 composite samples, or four composites of each of 11 pure varieties, grown over a period of two years. Similarly, adjusted lactic acid viscosity values for the same samples were related to cookie diameter. Variety means for each viscosity method also were correlated with the 11 variety means for cookie diameter. These relationships afforded a means of comparing the utility of the two tests for predicting cookie baking performance.

In order to determine the effect of protein content on viscosity, flours of 11 varieties ranging in quality from hard red to soft white were used. For each variety there was a total of 18 samples representing a wide range in flour protein content (from about 6% to 13.5%), and grown over a period of three crop years under widely different climatic and soil conditions.

Acid viscosity (no-time) as described in "Cereal Laboratory Methods" (1) was modified by using a single addition of 6 ml. *N*/1 lactic acid instead of several small increments.

The procedure used for the alkaline viscosity test was as follows: Adjust MacMichael Viscosimeter such that it is level, the cup rotates at a constant speed of 12 r.p.m., and the indicator needle points to zero when bob with No. 32 wire is placed in position. Weigh 20 g. flour (14% moisture basis) into a 500 ml. Erlenmeyer flask, add 60 ml. distilled water, and shake for one minute. Add four drops caprylic alcohol (to break the foam), shake slightly and pour into viscosimeter cup. Start motor, stir suspension vigorously with bob for 15 seconds, replace bob, and allow to turn slowly to maximum reading (e.g., 108°) while damping shaft between thumb and forefinger. Without stopping motor, return bob to zero position, remove, dispense 1 ml. of *N*/1 sodium bicarbonate solution, stir, replace bob and read maximum torque on wire (e.g., 63°). Repeat the operation a second time, adding another ml. of reagent. This second reading (e.g., 61°) is taken as the viscosity of the flour suspensioin. The entire operation should not take more than 4 minutes. There should be a large decrease in viscosity after the first ml. of alkali is added and a much smaller one after the addition of the second increment.

In the course of the development of the alkaline viscosity test, several items of note were observed. First, due to the low viscosity values obtained in alkaline media, it was necessary to use a finer wire (No. 32) than is generally used for acid viscosity in order to obtain the desired range. Secondly, it was found that the addition of all the

alkaline reagent at one time made accurate readings difficult because of excessive drifting. Dispensing the solution by parts aided materially in obtaining replicable values. Thirdly, viscosity values are a function of time after an initial lag period. The making of determinations with minimum delay and at comparable speed for all samples is thus imperative.

Replications usually checked within 2° MacM. for lower readings and within 4° MacM. for higher values. The usual precautions as to temperature of room, flour, and water should be observed. All determinations herein reported were made at 25°C.

Results

Statistical data relative to the relationship between alkaline viscosity values and flour protein content for each of 11 varieties are presented in Table I. Although all correlation coefficients are significant, their magnitudes indicate the operation of other variables such as modifications in quality within a variety due to season, etc.

TABLE I

CORRELATION AND REGRESSION DATA FOR ALKALINE VISCOSITY (Y) VS. PROTEIN CONTENT (X) FOR 11 VARIETIES GROWN OVER A PERIOD OF 3 CROP YEARS AT SEVERAL LOCATIONS AND REPRESENTING A WIDE RANGE IN PROTEIN CONTENT

Variety	n	r	Regression Equation $\hat{Y}=$
Kharkof	18	0.57*	7.84X + 20.63
Purkof	18	0.57*	7.23X + 46.83
Minturki	18	0.51*	7.02X − 3.01
Kawvale	18	0.74***	9.73X − 15.26
Clarkan	18	0.50*	5.25X + 30.49
Trumbull	18	0.71**	9.15X − 20.58
Fairfield	18	0.69**	6.30X − 4.73
Thorne	18	0.79***	6.48X − 10.08
Wabash	18	0.64**	3.86X + 1.38
Blackhawk	18	0.77***	5.14X − 14.97
Am. Banner	18	0.68**	4.45X − 4.49

*, **, *** indicate significance at the 5%, 1%, and 0.1% levels, respectively.

The slopes of the regression lines were averaged in order to obtain a mean regression for all samples. By this method, an adjusting value of 6.6° MacM. per per cent protein was obtained and used in adjusting alkaline viscosity values to 9% protein content for the composite

samples. Although the assignment of one adjusting factor for all varieties may cause some inequalities in those varieties with extremes in slopes, significant errors probably are not involved because the actual numerical adjustments for protein are relatively small. The no-time acid viscosity results have also been adjusted to 9% protein on the basis of factors[3] that varied from 19.6° MacM. per per cent

TABLE II

PROTEIN CONTENTS, COOKIE DIAMETERS, ALKALINE AND ACID VISCOSITIES OF 44 VARIETY COMPOSITES OF FLOUR AND THEIR 11 MEAN VARIETY COMPOSITES

Variety	Crop Year	Flour[1] Protein	Cookie Diam.	Alkaline Viscosity		Acid Viscosity	
				As Rec'd.	Adjusted[2]	As Rec'd.	Adjusted[2]
		%	*cm. x 2*	*°MacM.*	*°MacM.*	*°MacM.*	*°MacM.*
Kharkof	1945–EUN[a]	8.9	16.1	84	85	80	82
(K)	1945–VPS[a]	10.4	15.6	122	113	113	84
	1946–EUN	8.8	16.4	84	85	72	73
	1946–VPS	11.1	16.4	94	80	103	68
	Mean		16.13		90.75		76.75
Purkof	1945–EUN	8.9	15.7	99	100	85	87
(P)	1945–VPS	10.0	15.3	126	119	108	87
	1946–EUN	8.6	16.1	110	113	79	83
	1946–VPS	10.7	16.2	105	94	105	74
	Mean		15.83		106.50		82.75
Minturki	1945–EUN	8.8	17.0	59	60	58	60
(M)	1945–VPS	9.4	16.3	69	66	70	64
	1946–EUN	8.9	17.5	41	42	43	42
	1946–VPS	10.5	17.3	50	40	67	49
	Mean		17.03		52.00		53.75
Kawvale	1945–EUN	8.2	16.9	60	65	57	71
(V)	1945–VPS	8.9	16.2	73	74	70	72
	1946–EUN	8.9	17.2	41	42	43	42
	1946–VPS	9.8	17.0	61	56	70	60
	Mean		16.83		59.25		61.25
Clarkan	1945–EUN	9.2	17.0	74	73	63	60
(C)	1945–VPS	9.4	16.7	80	77	68	62
	1946–EUN	8.8	17.2	77	78	59	59
	1946–VPS	10.3	17.2	73	64	70	53
	Mean		17.03		73.00		58.50
Trumbull	1945–EUN	8.7	16.7	61	63	70	76
(R)	1945–VPS	9.7	16.3	83	78	94	80
	1946–EUN	8.8	17.3	48	49	62	63
	1946–VPS	10.3	17.1	59	50	91	69
	Mean		16.85		60.00		72.00

[3] Unpublished data, Soft Wheat Quality Laboratory, Wooster, Ohio.

TABLE II (Continued)

PROTEIN CONTENTS, COOKIE DIAMETERS, ALKALINE AND ACID VISCOSITIES OF 44 VARIETY COMPOSITES OF FLOUR AND THEIR 11 MEAN VARIETY COMPOSITES

Variety	Crop Year	Flour[1] Protein	Cookie Diam.	Alkaline Viscosity		Acid Viscosity	
				As Rec'd.	Adjust-ed[2]	As Rec'd.	Adjust-ed[2]
		%	*cm. x 2*	°*MacM.*	°*MacM.*	°*MacM.*	°*MacM.*
Fairfield	1945-EUN[3]	7.9	17.4	42	49	55	76
(F)	1945-VPS[4]	8.6	16.8	59	62	75	83
	1946-EUN	7.7	17.7	38	46	48	70
	1946-VPS	9.3	17.4	45	43	75	70
	Mean		17.33		50.00		74.75
Thorne	1945-EUN	9.0	17.1	45	45	58	58
(T)	1945-VPS	9.4	16.8	59	56	77	70
	1946-EUN	8.1	17.6	39	45	48	62
	1946-VPS	9.5	17.2	44	41	67	60
	Mean		17.18		46.75		62.50
Wabash	1945-EUN	8.7	17.6	34	36	45	49
(W)	1945-VPS	9.3	17.2	40	38	55	51
	1946-EUN	8.4	17.9	29	33	37	39
	1946-VPS	9.7	17.8	32	27	54	47
	Mean		17.63		33.50		46.50
Blackhawk	1945-EUN	9.8	17.6	34	29	49	40
(B)	1945-VPS	10.9	17.4	41	28	60	40
	1946-EUN	9.4	17.9	28	25	40	37
	1946-VPS	11.4	17.9	39	23	66	41
	Mean		17.70		26.25		39.50
Am. Banner	1945-EUN	8.1	17.1	37	43	39	51
(A)	1945-VPS	8.5	17.5	37	40	41	49
	1946-EUN	8.0	17.6	30	37	28	37
	1946-VPS	9.4	17.8	33	30	40	36
	Mean		17.50		37.50		43.25

[1] 14% moisture basis.
[2] Values adjusted to 9% protein content.
[3] EUN – Eastern Uniform Nursery Composite.
[4] VPS – Variety Protein Series Composite.

protein for Purkof to 8.9° MacM. per per cent protein for American Banner.

Table II presents protein contents, cookie diameters, and viscosity data for the 44 individual composites and their 11 mean variety composites; whereas Fig. 1 shows the relation between cookie diameter vs. adjusted alkaline and acid viscosity values for the individual composites of flour (top) and their 11 varietal means (bottom).

Significant improvements in correlation (Table III) are seen when alkaline instead of acid viscosity is used as a criterion of cookie baking quality. For example, the correlation data for the varietal means in-

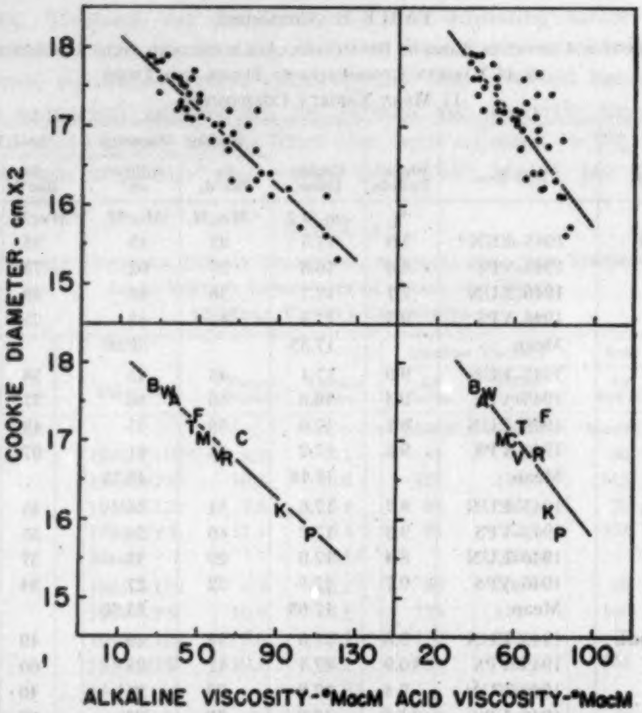

Fig. 1. Cookie diameter versus adjusted alkaline and acid viscosities for 44 variety composites of flour (top) and their 11 mean variety composites (bottom). See Table II for key.

TABLE III

STATISTICAL DATA FOR RELATIONSHIP BETWEEN COOKIE DIAMETER (Y) AND ADJUSTED ALKALINE AND ACID VISCOSITIES (X)

Relationship	n	r	Regression Equation $\hat{Y}$ =	Standard Error of Estimate Sy.x
				cm. x 2
Individual Composites				
Cookie Diam. vs. Alkaline Visc.	44	−0.93***	−0.024X+18.40	0.25
Cookie Diam. vs. Acid Viscosity	44	−0.81***	−0.034X+19.10	0.39
Varietal Means				
Cookie Diam. vs. Alkaline Visc.	11	−0.98***	−0.023X+18.35	0.13
Cookie Diam. vs. Acid Viscosity	11	−0.83***	−0.033X+19.04	0.35

*** indicate significance at the 0.1% level.

dicate that alkaline viscosity would accurately predict the cookie quality of 96 out of 100 samples; whereas acid viscosity would accurately predict the quality of only 69 out of 100 samples. These im-

provements in correlation also are reflected in the smaller standard errors of estimate involving alkaline viscosity (Table III, last column).

Discussion

Alkaline viscosity results within a variety, like mixogram area and acid viscosity, are influenced by protein content differences; whereas cookie diameter is affected only slightly by protein content. Thus, it is necessary to adjust the viscosity values to a constant protein content for intervarietal comparisons. For the sake of simplicity, and because extremes in protein content were not encountered for the composites, a mean regression value was used in adjusting all alkaline viscosity results to 9%. In common with other tests, however, it appears likely that the accumulation of more alkaline viscosity vs. protein content data will show that a fan shaped family of regression lines exists for different varieties of wheat, those with higher viscosities having the greater slopes.

The data presented in this paper indicate that the alkaline viscosity test has some advantages in evaluating the cookie baking potentialities of varieties of soft wheat flour. The improvement in correlation between cookie diameter and viscosity when the pH of the medium is changed from acid to slightly alkaline must be attributed to the fact that the relative rankings of varieties differ under these conditions. Thus, a variety which hydrates greatly in lactic acid may or may not absorb water to the same relative extent in a solution of sodium bicarbonate.

Although acid viscosity is a fairly good index of the cookie baking potentialities of wheat varieties, the alkaline test gives a more accurate evaluation of varieties. In addition, the differentiation or spread between varieties by alkaline viscosity is about twice that by the acid test.

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THE EFFECT OF THE CRUST ON THE STALING OF BREAD[1,2]

W. G. Bechtel, D. F. Meisner, and W. B. Bradley

ABSTRACT

Crumb compressibility, crumbliness, and swelling power changed in the same manner with age, regardless of whether bread was stored intact or with crust removed. Crustless bread remained at a constant moisture level whereas in the intact loaves, some moisture migrated from crumb to crust. Great differences in freshness were observed by a sensory perception panel after the second day. Crustless bread maintained its freshness while the intact loaves staled rapidly.

The nature of the changes which cause the staling of bread is still not understood, although the problem has been studied for approximately a century. This is not altogether surprising, for staling has never been defined in objective terms. In early studies of the phenomenon it was observed that certain physical changes occur in bread as it ages. The crumb becomes firmer and more crumbly, the water absorbing power of the crumb decreases, and often the amount of carbohydrate which can be extracted from the crumb by water, the so-called soluble starch, also decreases. More recent investigators have developed improved quantitative methods for measuring these changes and have usually assumed that by making such measurements they were measuring the staling of the product. It has long been known that these physical changes do not occur at the same rate. Katz (10) for example stated that crumb hardness, swelling power and the amount of soluble starch indicate that bread is stale when nine or ten hours old, while changes in crumbliness are not important during the first 24 hours. Thus, if such tests are considered as measures of staling, the conclusion may be drawn that the rate and extent of staling depend on the method used to make the test.

Actually staling is a term which indicates decreasing consumer acceptance of bakery products caused by changes in the crumb other than those resulting from action of spoilage organisms. The purpose of studies into the cause of the phenomenon has been to extend the period of time during which baked products are acceptable and re-

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[2] A report based on the data given herein was presented before the Thirty-seventh Annual Meeting of the American Association of Cereal Chemists, Dallas, Texas, April 1952.

garded as palatable by the consumer. Thus the test of fundamental significance is that of consumer evaluation. There has been an increasing realization in recent years that the consumer does not judge the freshness of bread by the arbitrary standards of physical laboratory tests (6, 4) but uses such subjective sensory perceptions as feel to the fingers, feel in the mouth, flavor, and odor. Decision as to the freshness or staleness of the product is based on a complex, conscious or unconscious evaluation of these perceptions. Bakery products are fresh as long as the consuming public judge them to be fresh, regardless of their age in hours, and regardless of the results of the usual laboratory tests. They are stale when the consuming public regards them to be so.

Recently Bechtel and Meisner (4) have reported results of sensory perception tests of staling of commercial white bread, in which a large, randomly-selected panel was used. The method of paired comparison of samples permitted quantitative evaluation of the rate and extent of staling which was reported in terms of a scale value. An interesting observation was that, although the sensory perception panel judged this bread to grow stale with age, and although there were corresponding changes in crumb compressibility, swelling power, and crumbliness, there was no significant change in the quantity of soluble starch or of its amylose or amylopectin content. Carson, Marnett, and Selman (9) have reported similar results for the soluble starch test, although the conditions of their tests differed from those referred to above. Since bread may stale, while the soluble starch fraction remains essentially unchanged, the conclusion may be reached that measurement of soluble starch is not a valid means of following the staling of bakery products.

Recently Bradley and Thompson (8) made a study of the effect of moisture migration from the crumb to crust of bread on crumb compressibility and crumbliness. These investigators compared the compressibility and crumbliness of the crumb of intact loaves stored in tightly closed tinned cans with the crumb of similarly stored bread from which the crust had been removed. In the intact bread there was a migration of moisture from crumb to crust throughout the four day period of the test. The crumb without crust maintained a constant moisture level. Crumb compressibility and crumbliness of the two kinds of samples changed in a similar manner with time, but the investigators noticed a decided difference in apparent freshness of the samples when judged by sensory perception methods. Bread stored with crust intact appeared to be less fresh than that stored for an equal time without crust.

The present study was undertaken to investigate this observation and to obtain further evidence regarding the validity of the physical laboratory tests of staling.

Materials and Methods

Preparation and Storage of Bread. For the experimental work bread was baked by the sponge-dough process, using the following formula:

	Sponge	Dough
	%	%
Flour	60	40
Yeast	2.0	
Arkady	0.5	
Malt flour	[1]	
Salt		2.0
Sugar (sucrose)		4.0
Nonfat dry milk solids		4.0
Lard		2.0
Calcium propionate		0.25
Water	[2]	[2]

[1] As determined by the Amylograph to give a reading of 500 Brabender units when 100 g. flour is suspended in buffered distilled water to a total of 450 ml. at pH 5.35 and heated at the rate of 1.5°C. per minute.
[2] As determined by the Farinograph and a preliminary baking test.

The calcium propionate was added to all the bread as a mold inhibitor to minimize the possibility of mold growth in the bread stored after removal of the crust.

As soon as the bread cooled sufficiently, the crust was carefully stripped from some of the loaves to a depth of half an inch, using a sharp, thin-bladed knife. This left the center crumb in a loaf approximately 2½ in. wide, 3 in. high, and 8 in. long. Precautions were taken against handling the crumb, to avoid contamination with microorganisms. Crumb from which the crust had been removed, and intact loaves were placed on waxed paper and immediately inserted in individual heat-sterilized, press-top tinned containers with tight-fitting lids. The cans were stored in a constant temperature cabinet maintained at 75°F. (24°C.) until required for use.

Laboratory Determinations. Moisture determinations were made by the A. O. A. C. method (1). Compressibility measurements were made upon three center slices, two inches thick, from each of two loaves of bread. The crust was cut from intact slices so that all measurements were made on pieces of crumb of the same dimensions. A

penetrometer of the type specified by the American Society of Testing Materials for petroleum products was used with a disk 3 cm. in diameter, in place of the cone. The disk, under a load of 215 g., was allowed to compress the crumb for 10 seconds. The average of the six measurements was taken as the compressibility. Crumb swelling power was tested by the method of Schoch and French (13). Crumbliness was determined on sixteen cubes of center crumb, 1 inch in dimension. The cubes were placed on a U. S. No. 4 sieve covered on top and bottom. They were then shaken at constant rate for 15 minutes in a mechanical shaker. Cubes and crumbs were weighed separately. Crumbliness was calculated as the percent of crumb which passed through the sieve.

Sensory Perception Evaluation of Staleness. In preparation for the sensory perception tests, sample loaves were removed from the cans shortly before the time of testing. The intact loaves and the crumb from which the crust had been removed were cut into slices half an inch thick. In the case of the intact bread the crust was then cut off so that all samples given the panel members were slices of crumb of identical size and shape. Samples were packaged at once in double-wall bags of moisture-proof cellophane, which were then heat-sealed. Each sample was coded with a two-digit number selected by chance, and each bag carried the sample number.

The panel of 100 members was composed of members of the student body of the School of Baking and staff members of the American Institute of Baking. These people were not trained, nor were they necessarily experts at judging bread quality. Each panel member was permitted to form his judgment of the samples individually, on the basis of flavor, odor, touch, appearance, or by any combination of sensory perceptions which he would normally use in judging the freshness of bread. Samples were presented one at a time so that preference was not a factor. Tests were conducted at the same time of day, and under uniform conditions of temperature, humidity, light, and surroundings.

The serial method of Martin (12) was used and the results were analyzed by the procedure which Beebe-Center (5) applied to Martin's method. The essential feature of the serial method is to restrict the possible judgments to one of two choices. Accordingly panel members were given blanks on which to write the sample number and their judgment of each sample as *fresh* or *stale.* No qualification of judgment was permitted. Beebe-Center extended Martin's method to permit quantitative evaluation of the results, by calculating the percent of the total panel which made each judgment. Applying this method

to the judgments of staling, the percent of the panel members who judged each sample to be fresh becomes the percent freshness of the sample.

Results

Typical data from physical laboratory tests are given in Table I. Migration of moisture from crumb to crust of intact loaves of bread has been observed by Bice and Geddes (6) and by Bradley and Thompson (8). In the present experiments moisture loss from the crumb of intact loaves is shown in column 2.. Migration of this moisture to the crust is indicated by the fact that the one-pound loaves of bread lost only 1.04 g., or 0.23%, in weight during the six day period. As shown in column 6 there was no significant change in the moisture of crumb stored without crust. The work of Bradley and Thompson was confirmed in the finding that there was no significant difference in crumb compressibility, crumbliness, or swelling power of samples of equal age of bread stored with crust intact and without crust. Such differences as appear in the table are generally within the limits of experimental error of these tests.

TABLE I

MOISTURE, COMPRESSIBILITY, CRUMBLINESS, AND SWELLING POWER OF BREAD STORED WITH OR WITHOUT CRUST AT 75°F. (24°C.)

Age sample	Bread with Crust				Bread without Crust			
	Moisture	Compressibility	Crumbliness	Swelling power	Moisture	Compressibility	Crumbliness	Swelling power
hr.	%	*mm. x 10*	%	%	%	*mm. x 10*	%	%
2	43.4	272	0.3	510	43.4	272	0.3	510
20	43.9	149	7.2	335	44.4	115	9.4	318
44	42.3	80	14.9	302	43.7	76	18.8	296
68	42.0	45	17.8	285	43.7	53	21.3	291
92	41.6	58	16.5	282	43.8	46	23.4	280
116	41.0	36	23.1	275	44.1	41	22.8	265
140	39.8	31	20.9	267	44.4	33	24.8	268

Sensory perception judgments of freshness are given in Table II. The two samples were judged equally fresh, 87%, at 20 hours. Again at 44 hours there was no significant difference and both samples were judged about 75% fresh. However at 68 hours bread stored with crust intact was 52% fresh while the crustless bread was 72% fresh, a difference which was shown statistically to be below the 0.5% level of significance. In succeeding 24 hour periods the bread with crust intact

diminished in freshness to 14% at 140 hours, while the crustless bread decreased in freshness only a small amount, to 63% fresh at 140 hours. Repetition of the experiments has confirmed the observations.

TABLE II

SENSORY PERCEPTION JUDGMENT OF FRESHNESS OF BREAD STORED WITH AND WITHOUT CRUST AT 75°F. (24°C.)

Age sample	Number of judgments	Percent freshness		Significance of difference in judgment
		Intact bread	Crustless bread	
hr.		%	%	
2	. . .	. . .	. . .	
20	95	87	87	Not significant
44	95	75	74	Not significant
68	95	52	72	Below 0.5% level
92	98	44	68	Below 0.1% level
116	98	38	68	Below 0.1% level
140	98	14	63	Below 0.1% level

Discussion

The data show lack of agreement between the physical laboratory tests and sensory perception judgment of freshness in two major respects. First, there is the failure of the physical tests to show differences between the crumb from the intact loaves and that from the crustless bread of the same age, in the period from 44 to 140 hours. During this period the sensory perception panel noted an increasing difference in their freshness. Second, approximately half of the change in crumb compressibility, about one-third of the total change in crumbliness, and three-fourths of the total change in swelling power had occurred when the bread was 20 hours old, when the sensory perception judgment was 87% fresh. That is, the panel considered the bread fresh, although the greater part of the changes in the properties measured by the physical laboratory tests had already occurred.

These results, together with those reported by Bechtel and Meisner (4) on soluble starch, lead to the conclusion that the common physical tests have little significance as measures of the bread-staling process.

They indicate that the presence of the crust is the cause of a large part of the staling of the crumb. This does not give a complete explanation of the staling process, however, for it does not explain the equal decrease in crumb freshness of intact and crustless loaves during the first two days. It is possible that this part of the staling process may be due to the large increase in firmness of the crumb which occurs during this period.

Three possibilities are suggested to explain the rapid staling of the intact loaves in the period from 44 to 140 hours. First, it may be due to loss of moisture by the crumb. Second, it may be due to undesirable flavors or odors absorbed by the crumb from the crust. Third, it may be caused by both moisture loss and undesirable flavor and odor.

The fact that crumb of crustless and intact bread staled at the same rate for the first 44 hours does not suggest which hypothesis is correct. If moisture loss to the crust is the cause of the latter difference, it would be expected that no variation in freshness would become apparent until after a sufficient differential in moisture developed between the two breads. The increase in this differential with time of storage is shown in Fig. 1. If the rapid staling of crumb of intact loaves is due to odors and flavors imparted by the crust, one would similarly expect that it would require time to develop differences sufficiently great to be detected.

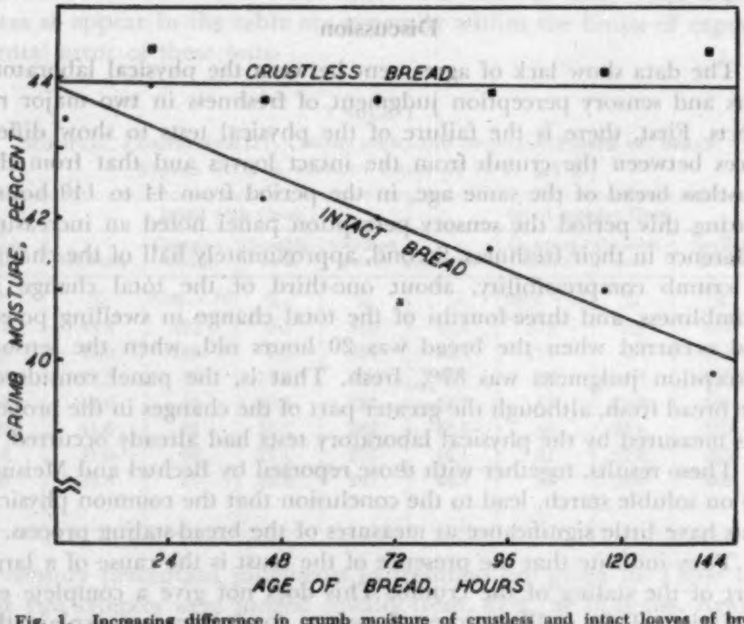

Fig. 1. Increasing difference in crumb moisture of crustless and intact loaves of bread with increasing time of storage.

Each hypothesis is in accord with some observations of staling phenomena. Boutroux (7) in 1897 concluded that the staling which occurred during cooling of bread was due to migration of moisture

from crumb to crust. He reasoned that, as the crust cooled first, its vapor pressure was lowered while that of the warm crumb was relatively high. Therefore moisture would "distil" from crumb to crust. He also attributed the softening of bread reheated in the oven to the distillation of moisture from crust to center, and observed that when a cylinder of crumb was heated in a closed container at 60°C. for 1 hour only the center crumb became soft, while that at the outside became hard and dry forming, in effect, a new crust. The authors have found that when crumb was heated for 24 hours by the method of Boutroux, to allow the cylinder of crumb to come to moisture and temperature equilibrium, the outer crust disappeared and the entire crumb was softened. Thus the softening of stale bread upon heating for a short time is probably not entirely due to moisture migration. Boutroux did not regard moisture migration to be a cause of staling of completely cool bread. He attributed staling under this condition to slow crystallization of what he termed "amylodextrin" from a hypothetical supersaturated solution which he believed to exist in bread. This view was not substantiated by later experiments of Lindet (11).

The second hypothesis is supported by the observations of Baker and Mize (3). These investigators showed that bread baked without a crust had a mild, yeasty flavor which became less pronounced on storage. Bread baked in the usual manner had a strong flavor and aroma due to the effect of high temperature on the crust. This aroma was also present in the crumb, and could be observed when the crumb was separated from the crust. On storage, bread baked in the conventional manner, developed a characteristic stale odor. Recently Baker (2) has suggested that the stale flavor of bread may be caused by oxidation of isoaldehydes (heat degradation products formed in crust during baking) to acids.

Investigations are now being conducted to test the validity of these hypotheses.

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COMMUNICATION TO THE EDITOR

Application of the Polaroid-Land Process to Radiographic Inspection of Wheat[1]

DEAR SIR:

At a time when the technique of X-ray inspection of grains for internal insect infestation, announced almost two years ago[2], appears to have gained considerable currency in industries handling and processing grain[3], there exists the general feeling that these industries would be more receptive to any technique which would even further simplify and expedite the radiographic inspection process. To this end Kansas State College and the Picker X-Ray Corporation have engaged in a cooperative study relating to the application of the Picker-Polaroid photographic process to the determination of internal infestation in grain. This process is based on the principle of the Polaroid-Land camera and requires no darkroom or processing of film in solutions. Approximately one to two minutes after an exposure is made, a positive print can be removed from the processing box in suitable condition for examination.

For normal use in medical radiography the packet of photographic material supplied consists of a sheet with an X-ray sensitive emulsion combined with a pod of developing chemicals, both contained in a light-tight black paper envelope to which is attached a sheet of specially prepared paper on which the positive image finally appears. The film packet is loaded into a special cassette (film holder) and the X-ray exposure is made in the usual way. Processing the exposed film is accomplished in a small automatic unit which can be located beside the X-ray machine. No darkroom or solution tanks are required.

In our application it was necessary to minimize the amount of radiation-absorbing material in the path of the X-ray beam. With the low excitation voltages required for the production of a suitable radiographic image of wheat, as little as 1/32″ of lucite absorbs out an appreciable amount of the beam intensity and 3/32″ of lucite almost completely absorbs the soft radiation. Consequently, we exposed the sensitive emulsion without using any cassette, by simply placing the packet in its black paper envelope on the exposure table and sprinkling a layer of grain on it. A lucite step-wedge made from 1/32″ thick

[1] Contribution No. 222 Department of Flour and Feed Milling Industries, and Contribution No. 28 Department of Physics, Kansas Agricultural Experiment Station, Manhattan, Kansas.

[2] Milner, M., Lee, M. R. and Katz, R. Application of X-ray technique to the detection of internal insect infestation of grain. J. Econ. Entomol. 43:933-935 (1950).

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lucite sheets cemented together also was placed on the film in order to better judge the contrast of any exposure made. Usable radiographs were obtained with 90 seconds of exposure at 16 kilovolts while exposures which we believe are as contrasty as could be used on a paper print were obtained with an exposure of 20 minutes at 13 kilovolts. The beryllium window X-ray tube had a molybdenum target and was operated at about 12 milliamperes in each case. Use of a tungsten target tube and the higher currents of which such a tube is capable, would materially decrease exposure time. Figures 1 and 2 representing different exposures, are reproductions of Polaroid radiographs of infested wheat taken in the manner indicated.

It may be stressed that the Polaroid process provides a positive print rather than the negative reproduction given by radiographs made with ordinary X-ray film. The reproduction with the Polaroid materials, while not providing the great detail appearing in a properly prepared radiograph, appears nevertheless to be entirely satisfactory for the inspection of grain for gross internal insect infestation.

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October 13, 1952

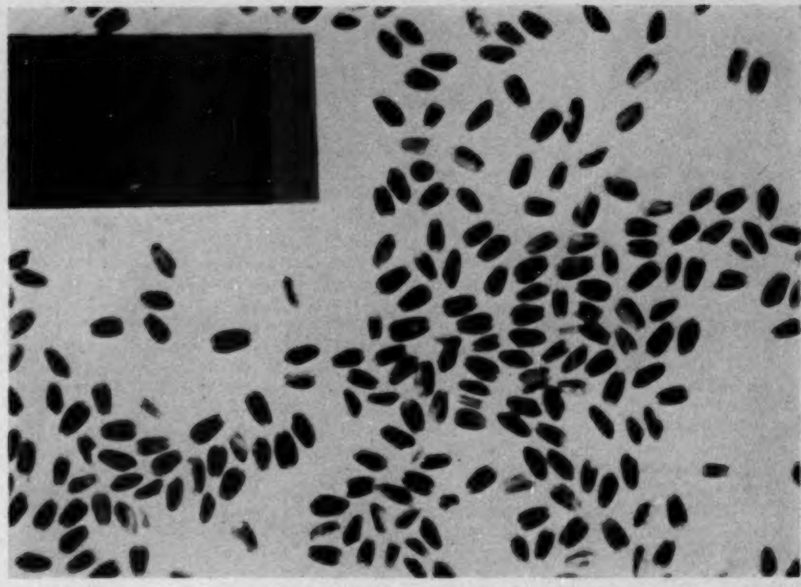

Fig. 1. Polaroid process radiograph of wheat infested with rice weevil, exposed for 90 seconds at 16 kilovolts and 12 milliamperes (Low contrast exposure), molybdenum target X-ray tube.

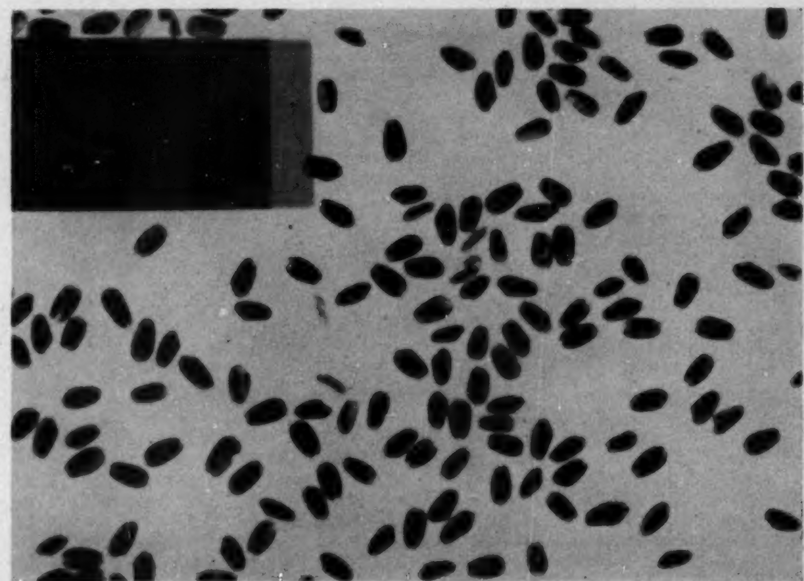

Fig. 2. High contrast exposure, similar to Fig. 1 but with 20 minutes exposure at 13 kilovolts and 12 milliamperes.

BOOK REVIEWS

Contributions of Browning Research to Ration Item Stability. Research and Development Associates, Food and Container Institute, Inc. 56 pp. paper bound. Chicago, Illinois, 1952. Price, $1.00.

In its search for methods of improving quality of rations for the Armed Forces, the Quartermaster Food and Container Institute at Chicago has given much attention to problems of stability. One important phase has been the deteriorative changes which occur during processing and storage of dehydrated foods, particularly those which involve the so-called Maillard or browning reaction. At a conference held in Chicago in February 1952, this subject was reviewed by several well known investigators. This booklet contains the reports presented.

Colin Lea of Cambridge, England described the results of his studies of the reactions which occur between proteins and reducing sugars in the "dry" state. He cited experiments illustrating the role of the ε-amino group of lysine and the importance of relative humidity.

Gordon MacKinney of the University of California reviewed the high lights of his study of the effect of sulfur dioxide in retarding nonenzymatic browning in dried fruits, and discussed the role of sugar and the source of carbon dioxide.

C. D. Hurd of Northwestern University and M. L. Wolfrom from Ohio State University critically reviewed their studies on model systems designed to reveal the complex reaction mechanism which accompanies browning.

W. E. Pyke of Colorado State College summarized his work on the prevention of browning in potatoes by removal of the reactants; and C. O. Guss of the same institution reported some interesting observations about the inhibitory effect of sulfhydryl groups.

J. C. Speck, Jr. of Michigan State College described his work on model systems, giving emphasis to the catalytic function of amino acids.

The pamphlet includes the discussion which followed the presentation of these reports and closes with a review by H. S. Olcott of the Western Regional Research Laboratory of the browning reaction studies in relation to product applications.

While the booklet makes no pretense of offering a complete review of the research carried out on the browning reaction, it presents an interesting and stimulating discussion of an important aspect of modern food technology.

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Biometric Analysis — An Introduction. By Allan Treloar, v + 251 pp. Burgess Publishing Co., Minneapolis, Minnesota, 1951. Price, $4.50.

This book is an experimental revision of the earlier publication *Elements of Statistical Reasoning* by Professor Treloar. The first eight chapters discuss such topics as: numerical description, variation, the law of frequency distribution, typical values, amount of variation, form of variation, the normal frequency distribution and cumulative frequency. The errors of random sampling are discussed in chapter nine with adequate diagrams and notes on the sampling distribution of means and standard deviations. This is followed by a chapter on significance of differences. Three chapters are devoted to proportional frequency and probability, frequency ratio in vital statistics, and sampling errors of proportions. The volume concludes with chapters on the chi-squared criterion, bivariate distributions and the correlation coefficient, rectilinear regression and residual variation, sampling errors of the correlation coefficient, differences between correlated pairs and chi-squared as a test of significance. Both the style of writing and the wealth of diagrams in the text reflect the work of a man who has had years of experience

in teaching students from many disciplines. Professor Treloar is to be commended upon introducing exercises for home study which did not appear in the Wiley edition of "Elements of Statistical reasoning".

The subject matter would be of use in a one semester course in elementary statistics. The first two chapters fortified with examples and exercises from the scientific fields might well be used in orientation courses given at many universities. Such examples might illustrate common fallacies in reasoning and show the part played by biometrics in modern science. A criticism of selected quotations could form the basis of certain problems that could be included at the end of chapter one.

It is regrettable that the author did not include in his chapter on numerical description of data some account of decimal accuracy and the problems confronting a worker who wishes to round off figures. The inclusions of a few examples in this descriptive chapter would have improved it.

Chapter three contains a very interesting account of different frequency distributions. A very clear and concise account of the setting up of class ranges and class centers is given. At the end of chapter five on pages 59 and 60 the quotation ". . . the logic which leads to this question being raised would seem to require that the question be answered in the affirmative" occurs. This sentence would imply that it is by logic alone that one could derive the fact that the division of the sum of squares by $N - 1$ be used to estimate the variance of the population or supply. The division of $N - 1$ is derived entirely from mathematical considerations and it would be clearer to state the conclusions reached by mathematical treatment earlier in this discussion.

The modern research worker now expects to find even in an elementary text some guidance on problems which daily confront him. No treatment is given in this text on the determination of sample size, the analysis of variance and curvilinear regression. Heavy weight is still being given to the correlation techniques and the distribution of the correlation coefficient. Less space could have been devoted to the distribution of means and more given, for example, on the analysis of variance. The author has expanded his former treatment of chi-squared but no reference is made to Mainland's tables.

The volume concludes with an index which is a model of completeness.

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A Review of Flour Milling Processes. By J. H. Scott. Scond Edition Revised. Chapman and Hall, Ltd., London, England, 1951. Price, 55s.

By comparison with existing tevxtbooks on flour milling, *A Review of Flour Milling Processes* is recommended because of the extensive references to the available literature. The organization of the book is familiar. The first part concerns wheat, its selection and preparation for milling, and the second part describes the milling process itself. Since the author is English, the book reflects the English viewpoint of milling, but reference is frequently made to the literature of other countries. The author endeavors to extract from the literature data and formulas applicable to the subject being discussed. While the reader may not find the solution to his own particular problems, he may be provided with ideas that suggest ways and means of accomplishing the desired results.

To illustrate, in the chapter on Machines Used in Wheat Cleaning, curves are shown indicating the capacity in bushels of grain per hour relative to the speed and stroke of the sieve. Of interest to the engineer is the author's derivations of the predicted sieve capacity from the relationship between stroke, speed and coefficient of friction between the grain and the seive. Whether or not any particular investigator may agree with the assumptions and the derivation, it at least provides one of the few available analytical approaches to the problem of sieve capacity.

For the reader who is interested in practical information for immediate application, most of these subjects are also discussed from the standpoint of workable practice.

Chapter 4 discusses the behavior of moisture in wheat as an introduction to Chapter 5 on wheat conditioning by various methods. An excellent review of the literature is given in Chapter 4, although the information available on equilibrium moisture contents and the hygroscopic capacities of wheat may leave the reader in some confusion. The author's discussion of the mode of entry of water into wheat seems to indicate greater agreement than in the case of equilibrium moisture content. Much of this information was contained in an article widely read in this country called "Observations on the Rate of Penetration of Water into the Wheat Grain," C. R. Jones, *Milling*, July 23, 1949. This same article was also republished in the Milling Production Section of the *Northwestern Miller* in September, 1949, for readers in the United States.

In the succeeding chapter on conditioning, the author discusses the effect of temperature upon the milling and baking character of the wheat. Research by Geddes is cited in regard to the effect on hard red spring wheat of various temperatures. The general conclusion has been that the improvement, due to heat treatment, was less than that obtained by the use of chemical improvers. The author provides an interesting summary of the physical-chemical changes associated with hot conditioning. In brief, his conclusions indicate that conditioning to modify the baking characteristics of the flour milled from wheats is desirable only in order to break down extremely strong flours for biscuit or cracker uses, or for toughening flour milled from extremely weak wheat.

Throughout Section 2 on the subject of the milling process itself, the author continues to provide a theoretical analysis of the various steps in milling. Again, this seems to be a unique feature of the book. While Scott's review of the theoretical estimation of roll surface required for the various breaks may not fit practice in all parts of the world, it as least provides a basis of analysis which can be modified to suit the particular conditions involved. The chapters on scalping, grading and purification are simply descriptive, although one interesting study is reported, showing the effect on purifier performance of variations in rate of feed. The author is also to be commended on tracing more clearly than has been done in previous texts, the course taken by the germ stocks. The chapter on the reduction system includes a brief analysis of the phenomena of grinding and presents a practical investigation for comparison with the theoretical analysis. The practical miller will be intrigued by the discussion of scrapers and brushes for use with reduction rolls.

For those who are particularly interested in experiments on grinding, the author presents an entire chapter on experiments dealing with reduction roll grinding. These include a series of tests showing the relationship of roll setting to release. Experiments are also described in which the effect of alterations on the coarse middlings roll are studied in terms of the milling results obtained on other rolls in the mill. In brief, it was concluded that experiments of this type were of value only to the particular mills where the experiments were run.

Other chapters of interest discuss flour extraction, mill stock moisture and its control, aspiration and dust control, and flour bleaching and improving processes, which gives a brief description of many rare as well as common methods of flour treatment. In conclusion, the cereal chemist, miller and student will find Scott's new edition a worthwhile reference.

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Soil Physical Conditions and Plant Growth. Edited by Byron T. Shaw, 491 pp. Academic Press, Inc. New York. 1952. Price $8.80.

The volume is No. II in a series of monographs prepared under the auspices of the American Society of Agronomy. The present volume consists of five chapters and an epilogue by the Editor.

Chapter 1 is divided into two parts: I, Soil as a physical system by Lyle T. Alexander and II, modifying the physical properties of soil, by H. E. Middleton. This places together excellent discussions of the physical components of the soil and methods of modifying them.

Mechanical impedence and plant growth are discussed by J. F. Lutz in Chapter 2. The chapter discusses the effects of different sized soil particles, of soil structure, soil crusts and subsoil characteristics. The chapter brings together information on mechanical impedance not commonly found in text books.

Chapter 3 by L. A. Richards and C. H. Wadleigh deals with soil water and plant growth. It considers both the theoretical and practical aspects. Some of the latter relate to drainage and irrigation and the measurement of different forms of moisture. It also includes an excellent discussion of soil moisture in relation to plant growth.

Soil aeration and plant growth by M. B. Russell is considered in Chapter 4. Consideration is first given to soil processes and properties as affected by aeration and this is followed by a discussion of aeration and plant relationships such as aeration and root morphology and the effect of aeration on nutrient absorption. The chapter is a fine contribution on the subject.

Chapter 5, soil temperature and plant growth, by S. J. Richards, R. M. Hagan and T. M. McCalla is a timely one. It brings together all recent information on the subject and should be of great value to agronomists, plant physiologists, plant pathologists, and soil scientists.

Each chapter is followed by a long list of references dealing with the subject matter covered. These alone are valuable contributions and add much to this excellent volume.

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Food and Population and Developmnt of Food Industries in India. Symposia sponsored by Central Food Technological Institute. Mysore, India xv+357 pp. Wesley Press, Mysore, India, 1952. Price, Sh. 10/–.

This book is the outgrowth of two symposia sponsored by the Central Food Technological Institute, Mysore, India in May 1951, the purpose of which was to consider the ways and means of solving the critical food shortage now facing the population of India. This book contains the papers read and discussed by about 50 participants representing various government agencies, universities, institutes, and food industries of India.

The first part of the book contains the papers presented in the symposium entitled "Food and Population" and deals with the serious problem created by the failure of food production to keep pace with the steady growth of the population in India. If the existence of such a problem were not obvious at the outset, it certainly became so as each succeeding speaker presented statistics relating to population growth, death rate, birth rate, longevity, agricultural productivity, etc. There was no dearth of suggestions regarding the corrective measures that might be instituted. These included artificial restriction of the population by birth control, increasing the land available for cultivation by hydroelectric and irirgation projects, more intensive cultivation of the land presently arable, government subsidies to the farmer, and the education of the public to the use of foods which are not in short supply. Desirable as these long term measures may be it was generally agreed that there was an urgent need for immediate remedial steps of which the most promising appeared to be the use of subsidiary food items such as various tubers (tapioca, sweet potato, and arrowroot), yeast, peanuts, and fish.

The second part of the book consisted of papers presented in the symposium

entitled "Development of Food Industries in India". A wide variety of food commodities was represented including canned fruits and vegetables, biscuits, chocolate, confectioneries, sugar, flour, yeast, and edible oils. In general each speaker described the present status and future potentialities of his particular industry. All were in agreement that although India had made great strides in food technology, much remained to be accomplished. It was stressed that the extent to which the present handicaps could be overcome would depend to a large measure on the assistance and cooperation given by the proper governmental authorities.

Specific recommendations embodying many of the views expressed in the symposia were delineated by a special committee and are presented in the appendix. Any attempt to appraise the real value of this book must await the unfolding of future developments in India's efforts to cope with its food crisis.

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Chemistry of Food and Nutrition. By Henry C. Sherman. Eighth edition, viii + 721 pp. The Macmillan Co., New York, 1952. Price, $6.00.

Marked advances in nutrition have taken place since 1946 when the previous edition of this book appeared. In order to keep pace with these latest developments, every chapter in this book has been revised, several of them rewritten, and a new chapter on folic acid, vitamin B_{12}, and the citrovorum factor has been inserted. In general, however, the same basic approach which has characterized previous editions has been followed. A brief consideration of the chemical aspects of carbohydrates, fats, and proteins serves to introduce the main subject matter which appears in a sequence that closely parallels the chronological development of the science of nutrition, namely (1) the energy aspects of nutrition, (2) the proteins and their amino acids, (3) the mineral elements, and (4) the vitamins. These chapters, comprising about 75% of the book, are so written, however, that they may be studied in any sequence desired. The final quarter of the book is devoted to the practical application of nutritional knowledge to the health and well-being of the individual and the community. Considerable prominence is given to a consideration of such items as the quantitative requirements for nutrients (with particular stress on the beneficial effect of more than minimal-adequate levels of intake of such nutrients as calcium, ascorbic acid, riboflavin, and vitamin A), their distribution in natural foodstuffs, and the economic aspects of proper nutrition. Included in the appendix are tables giving the proximate composition, energy value, mineral and vitamin content of a wide variety of foods.

Although the author makes it clear from the outset that no attempt will be made to present any appreciable amount of material belonging to the fields of organic chemistry and biochemistry, it appears that he has been overly conscientious in this respect. Many instances could be cited where the substitution of descriptive detail for structural formulae or equations has detracted considerably from the clarity of the presentation. Attempts to describe the simplest of metabolic reactions without the use of formulae or equations may be likened to a word description of a painting. Biochemistry is so much a part of the science of nutrition that it is folly to attempt to maintain a rigid dividing line between these two sciences. Moreover, if such a distinction were attempted, does the simple omission of a structural formula constitute a proper respect for the boundaries of organic chemistry or biochemistry?

One glaring error was observed: on p. 17 maltose is referred to as the "milk sugar". What might be considered an error of omission was the author's failure to consider in any detail in chap. 31 (entitled, "Causes and Extent of Variations in the Nutritive Values of Foods") the loss of nutrients which occur during cooking or processing.

The real value of this book lies in the fact that it places emphasis on the application of the science of nutrition to everyday living. It should thus contribute substantially to the knowledge required by those who will be concerned with the maintenance of the health and efficiency of the community.

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Cereal Chemistry

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

Cereal Chemistry gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

The papers must be written in English and must be clear, concise, and styled for Cereal Chemistry.

Manuscripts for publication should be sent to the Editor in Chief. Advertising rates may be secured from and subscriptions placed with the Managing Editor, University Farm, St. Paul 1, Minnesota. Subscription rates, $11.00 per year. Foreign postage, 50 cents extra. Single copies, $2.50; foreign, $2.60. Back issues, $3.00.

SUGGESTIONS TO AUTHORS

General. Authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* 6:1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Editorial Style. A.A.C.C. publications are edited in accordance with *A Manual of Style,* University of Chicago Press, and *Webster's Dictionary.* A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used in following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10° C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B + C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

For more detailed information on manuscript preparation see *Cereal Chem.* 30: 69–70 (1953).

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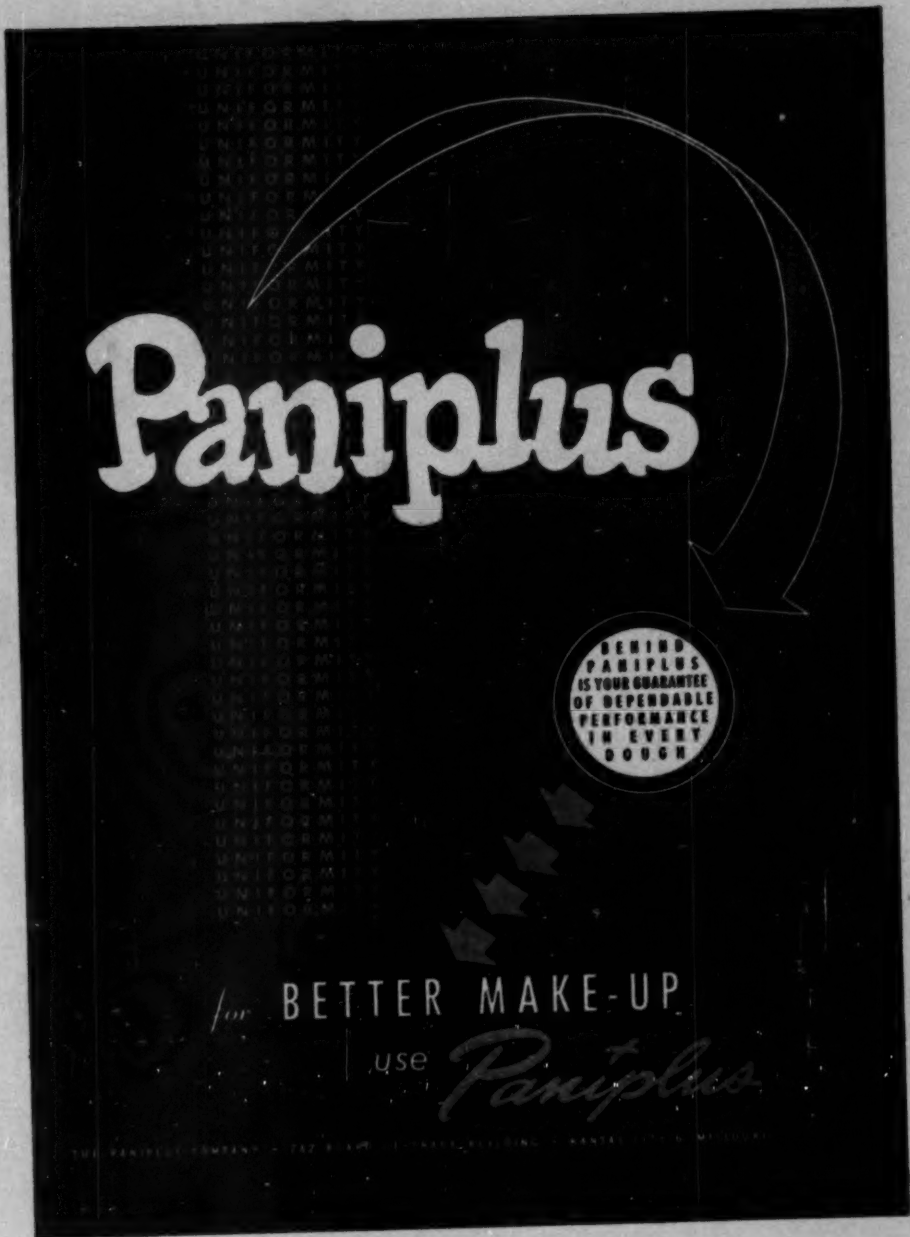

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